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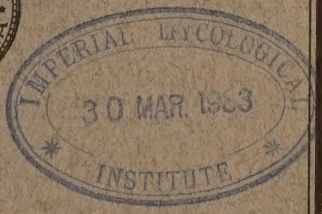
Root-Rots of Wheat

A. W. Henry
Division of Plant Pathology and Botany



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ROOT-ROTS OF WHEAT

By A. W. Henry[1]

INTRODUCTION

Root-rots of wheat and other cereal plants have been known for several years. Bolley was perhaps the first to emphasize the importance of these diseases in this country. He considered that root-rots were caused by several imperfect fungi which tend to accumulate in the soil as a result of more or less constant cropping to one kind of grain. In his estimation, root-rots were chiefly responsible for the unproductiveness of many of the wheat lands of the Northwest, rather than depleted soil fertility or toxic substances in the soil. Other workers at the Minnesota Agricultural Experiment Station and elsewhere have since corroborated many of Bolley's ideas.

As most of the publications on root-rots of wheat deal with them only incidentally, it seemed desirable to investigate them in particular, combining in this study both a comprehensive survey of possible root-rotting fungi and controlled inoculation studies to determine their relative pathogenicity. The plan followed has been to isolate fungi from as many sources as possible but particularly from such as are intimately associated with wheat rots in nature. Extensive inoculations were made and the most destructive and less familiar pathogenes were studied in greatest detail. While it is possible that micro-organisms other than true fungi may cause root-rots of wheat, only the latter were studied by the writer.

The roots of the wheat plant are very important in the economy of the plant, as they determine in large measure what the development of the above-ground parts shall be. Livingston (45), for instance, found that stunted wheat tops were associated with poor root development. Root-rots inhibit root growth, destroy absorbing cells, and by attacking the vascular system prevent the transportation of water and food materials. They consequently cause poorly developed tops.

[1] The writer gratefully acknowledges the helpful advice and criticism of Dr. E. C. Stakman, under whose direction the work was done. He also desires to express his appreciation to Dr. Louise Dosdall, Dr. J. G. Leach, and others to whom he is indebted for assistance of various kinds.

HISTORICAL

There is a comprehensive literature on foot- and root-rots of small grains, but there is comparatively little definite information on root-rots of wheat. The occurrence of fungi in association with rotted cereal roots is mentioned by several workers, but only rarely are inoculation experiments reported, or is any proof of the parasitism of the fungi given. There are, however, a few exceptions.

Root-rots of wheat are frequently attributed to species of *Fusarium*. For instance, Bolley (7) claims that *Fusarium spp.* are important root-rotting organisms of wheat and other cereals. Various European workers, Ihssen (34), Mortensen (52), and Schaffnit (68), for example, state that *Fusarium nivale* Ces. causes root-rot, foot-rot, and seedling blight of cereals. Stakman (75) found *F. culmorum* (W.G.Sm.) Sacc. very destructive to the roots of durum wheat seedlings in agar cultures. Atanasoff (1) considers that *Gibberella saubinetii* (Mont.) Sacc. (*Fusarium graminearum* Schwabe) is one of the chief causes of root-rot of cereals in the United States. However, he states that according to his observations, *Fusarium herbarum* (Corda) Fries, *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W.G. Sm.) Sacc., and *F. culmorum* (W.G. Sm.) Sacc. *var. leteius* Sherb., are as important as *Gibberella saubinetii*. *Fusarium arcuosporum* Sherb., *F. scirpi* Lamb. and Fautr., *F. solani* (Mart. pr.p.) Ap. and Wr., *F. arthrosporioides* Sherb., and *F. redolens* Wr. and also mentioned as being not unimportant in the root-rot problem. In a more recent paper (2), however, he mentions only *G. saubinetii, F. avenaceum,* and *F. herbarum* as causing root-rots of wheat but says that *F. culmorum* attacks the young seedlings in the same way as does *G. saubinetii*. He reports the occurrence, altho rarely, of *Calonectria graminicola* (Berk. and Brm.) Wr. (*F. nivale*) in the United States. An extensive bibliography of the literature on the *Fusaria* of cereals is given.

For several years Bolley (7, 8) has reported that species of *Helminthosporium* are destructive pathogenes of wheat roots in North Dakota. Johnson (38) used *Helminthosporium gramineum* Rabh. (more probably *H. sativum* P.K. and B.) in inoculation experiments. The roots of wheat plants from inoculated seed were discolored near the root crown and were also much shorter and less vigorous than those of check plants. Drechsler (20) cites papers by Bassi and Palm which report the occurrence of *Helminthosporium gramineum* Rabh. on wheat in Italy and Java, respectively. According to Bassi a decortication of wheat roots is caused by this fungus. Drechsler, however, points out the similarity of the symptoms produced to those caused by *H. sativum* and infers that the latter fungus was probably mistaken for *H. grami-*

neum by both workers. Stakman (75), in 1920, reported that the roots of wheat plants in Minnesota were frequently infected with a species of *Helminthosporium* similar to or identical with *H. sativum* P.K. and B. Such roots were partially or entirely brown and often developed loose cortices. Similar symptoms were produced by artificial inoculations. The writer isolated a *Helminthosporium* (tentatively determined as *H. sativum*) from wheat, rye, and barley, in Western Canada.[2] Artificially inoculated seeds of wheat, rye, and barley developed badly diseased seedlings in test-tube cultures, both roots and tops being affected. Oats were only slightly injured. In pot cultures similar results were obtained, but the disease in these tests was most noticeable as a foot-rot and a leaf-spot of the seedling leaves. *Helminthosporium* root-rots were reported from New York by Kirby (83) and from Idaho by Hungerford (83), in 1921, and by Raeder (62) in 1922. Hamblin (28) described abnormal roots as one of the symptoms of a disease of wheat caused by *Helminthosporium* in Australia. He considered this disease more serious than the true take-all. Christensen (13) was able to infect 98 species of grasses with *H. sativum*. Wheat, barley, and rye were the most susceptible cereals, while oats was either immune or highly resistant. All parts of a susceptible host, including the roots, were attacked. Both primary and secondary roots were severely rotted. Kanred wheat[3] C.I. 5146, was quite resistant to root infection.

Dosdall (19) studied the factors influencing the pathogenicity of *H. sativum*, giving particular attention to root- and foot-rots. She found that wheat and barley roots developed rather limited local lesions which killed the root tips or cut off portions or even whole roots when the lesions occurred back of the tips. In general, she found that the disease caused greatest injury when conditions for host development were unfavorable. Stevens (78, 79, 81) studied the relation of a species of *Helminthosporium* (of the *H. sativum* group) to a foot-rot of wheat in Illinois. He (79, 81) showed by artificial inoculations that it was a root parasite also, but investigated this phase of the disease only incidentally. Weniger (86) found a fungus similar to *H. sativum* causing lesions in the cortex of both the roots and the shoots of young seedlings, from black-pointed wheat seeds.

Numerous investigators have reported a discoloration and rotting of the roots as one of the symptoms of the true take-all disease of wheat. Stevens (77), in an extensive historical and bibliographic sketch on foot-rot of wheat, cites numerous papers in which mention is made of the blackening and death of wheat roots attacked by *Ophiobolus;* for instance, those of Frank (1894-95), Mangin (1898-99,

[2] Unpublished thesis, University of Saskatchewan, May, 1920.

[3] C.I.=Cereal investigations accession number of the Bureau of Plant Industry, U. S. Dept. of Agr.

1914), McAlpine (1902), Richardson (1910), Eriksson (1912), and Spafford (1917). *Leptosphaeria* also is said to cause a similar brown or black discoloration of the roots (Mangin, 1914). More recently Kirby (41) described the symptoms of the disease as they occur in this country. He pointed out that *Ophiobolus cariceti* (B. and Br.) Sacc., is confined to the roots and lower internodes of the host, where a pronounced discoloration occurs. Rosen and Elliot (63) believe that *O. cariceti* attacks only weakened plants.

In 1919 Selby and Manns (73) reported a new anthracnose disease of cereals in Ohio, caused by *Collectotrichum cereale* Manns. This fungus was said to be parasitic on the roots, culms, blades, and spikes of rye, and on the roots, culms, and blades of wheat, oats, barley, and several grasses. The pathogene, however, attacked wheat roots less severely than those of rye. Bolley (7, 8) has frequently reported *Colletotrichum* as one of the genera responsible for soil sickness and root-rots of cereals in North Dakota. Stakman (76) showed that *Colletotrichum phomoides* (Sacc.) Ches., isolated from a diseased tomato, was somewhat parasitic on the roots of wheat and oats, but she did not consider that it would be destructive in the field.

Bolley (7, 8) stated that *Alternaria* often caused decortication of wheat roots. *Macrosporium* was reported several times on wheat roots but later work indicated that this was mostly *Alternaria*. Johnson (38) inoculated wheat seeds with *Alternaria* but no root infection resulted. Stakman (76), however, isolated parasitic strains of *Alternaria* from cereal seeds and from the soil. These strains readily infected wheat roots both in agar cultures and in sterile sand. Stevens (81) inoculated wheat seedlings in rag-doll germinators with an *Alternaria* obtained from wheat seeds. He observed that the hyphae penetrated the cells of the root cortex and caused a slight browning.

Rhizoctonia has been found several times on cereal roots. Peltier (59) found it on corn roots in the field in Illinois, and lists its occurrence on corn in two other states and on side oats from Idaho (reported by Temple). He also cites a paper by Reuter, who studied a root-rot of rye caused by *Rhizoctonia* in Finland. Bessey (82) reported a *Rhizoctonia* root-rot of wheat in Michigan, occurring especially in heavy soils. Piper and Coe (61) list a large number of susceptible lawn grasses. Stevens (81) found *Rhizoctonia* on the roots and stems of wheat in Illinois. Stakman (76) isolated *Rhizoctonia* from the underground parts of rye, wheat, and oat plants in the field in Minnesota. A few artificially inoculated durum wheat roots became infected, but the tissues were apparently not disorganized.

In addition to the fungi previously mentioned, Stakman (76) showed that the following are parasitic on the roots of durum wheat: *Botrytis sp.*

from rye, *Gliocladium sp.*, and *Tilachlidium sp.* *Sclerotium rolfsii* Sacc. also was capable of attacking the base of the culms of durum wheat seedlings and of spreading into the roots after the tissues had become weakened. The latter fungus has also been reported by Godfrey (26) as the cause of small brown lesions on wheat crowns. The following fungi were also used by Stakman in inoculation experiments on durum wheat seedlings, but they were not parasitic on the roots: *Acrostalagmus albus* Preu., *Aspergillus niger* Van Tiegh., *Botrytis cinerea* Pers., *Botrytis fascicularis* (Cda.) Sacc., *Cephalothecium roseum* Cda., *Cladosporium cucumerinum* E. and A., *Cladosporium fulvum* Cke., *Monascus purpureus* Went., *Penicillium chrysogenum* Thom., *Rhizopus nigricans* Ehr., *Sclerotinia libertiana* Fel., *Sphaeronema fimbriatum* (E. and H.) Sacc., *Vermicularia circinans* Berk., *Verticillium candelabrum* Bonord., and *Sporotrichum poae* Pk.

Chen (12) found a sterile fungus with a septate mycelium which attacked the roots of wheat seedlings in test-tube cultures.

It is interesting to note that species belonging to at least twelve genera of fungi have been reported in the literature as parasitic on wheat roots.

ROOT SYSTEM OF THE HOST

The development and morphology of the wheat root system is described in detail by Percival (60). The following is based largely on his description. Two distinct set of roots are produced, the seminal and the adventitious. The former arise from the hypocotyl and begin to develop when the grain germinates. Eight seminal roots in all may be formed. They are slender, uniform in diameter, develop a number of fine lateral branches, and bear a very abundant covering of root hairs for some distance back of the root tips. These die off and new ones are formed near the growing point as the roots elongate. The seminal roots are apparently of most importance during the seedling stage of the plant, supplying it with water, but seem to remain functional throughout its life.

A considerable interval, often several weeks, may intervene between germination and the development of the adventitious or permanent roots. These are essentially fibrous. They arise in pairs from the basal nodes of the primary axis, usually just below the surface of the soil regardless of the depth at which the seed is planted. Succeeding shoots develop their own systems of permanent roots in a somewhat similar manner. The permanent roots are larger in diameter, longer, branch more profusely, and are more numerous than the seminal roots. They are usually most abundant in the upper foot of the soil but some extend four or five feet into the soil and to even greater depths.

METHODS AND MATERIALS USED IN THE ISOLATION OF FUNGI

The isolation of fungi was begun in January, 1921, and continued for approximately a year and a half. Cultures were secured from the seed, roots, and culm bases of wheat, from the corresponding parts of other cereals commonly grown in rotation with wheat, from the soil, and a few from other sources.

Between 500 and 1000 cultures were isolated. This number, however, was reduced to about three hundred by discarding duplications and retaining only those which were different or which came from different sources.

ISOLATIONS FROM SEED, ROOTS, AND OTHER PLANT PARTS

Wheat seed was obtained from eighteen states of the United States, from three provinces of Canada (Manitoba, Saskatchewan, and Alberta), from Mexico, and from the Argentine. Most of it was commercial grain grown in 1920, and much of it was obtained through the courtesy of the Washburn-Crosby Milling Company, of Minneapolis. Professors G. H. Cutler and W. P. Fraser each kindly sent samples from Alberta and Saskatchewan, respectively.

The seeds were surface sterilized at first by immersing them for ten minutes in a 1 to 1000 corrosive sublimate solution, followed by a thoro washing in sterile distilled water. This was a very time-consuming operation when a large number of samples had to be handled. A quicker method was therefore adopted which consisted of dipping the seeds in 70% ethyl alcohol, then immersing them in corrosive sublimate solution, 1 to 1000, for from 3 to 5 minutes, then dipping them again in 70% alcohol and plating the seeds directly on potato dextrose agar in petri dishes. This method was also used for other plant parts, and is the same as that used by Christensen (13) except for the strength of alcohol. Roots and underground parts were then frequently plated on acidified agar to prevent the growth of bacteria. The fungi were allowed to develop at room temperature for a few days and then transfers were made from the borders of colonies to slants of potato dextrose agar in test tubes.

ISOLATIONS FROM THE SOIL

Numerous isolations of fungi were made from "sick soils," particularly from the three following: (1) A soil from the plant pathology field plots, University Farm, St. Paul. This soil had been used for several years as a disease garden in the study of imperfect fungi on cereals. (2) Soil from a continuous-wheat plot from the rotation plots of the agronomy department, University Farm. Wheat had been

grown on this plot every year since 1893, except 1915 and 1916, when it was in corn in order to control wild oats. (3) A continuous-wheat plot soil from the Dominion Experimental Farm at Brandon, Manitoba.[4] This plot was first sown to wheat in 1911 and had grown eleven successive crops of wheat when the samples were taken. The first samples from these plots were taken during the last week of September and the first week of October, 1921. Additional samples were obtained from the St. Paul plots at intervals during the following summer.

In order to be reasonably sure that the fungi isolated were largely from the soil and not surface contaminations from the air, precautions similar to those used by Jensen (35) were observed in taking samples in the field.

In the collection of samples at St. Paul, small quantities of soil were taken from several locations on each plot, and these were later mixed together before isolations were made. This was done in order to secure a more representative sample of the soil of each plot.

Fungi were isolated directly from the soil by two methods: (a) A dilution method in which approximately one gram of soil was transferred to a flask containing 100 cc. of sterile distilled water. This was thoroly shaken and a platinum loopful of this extract, or of further dilutions, transferred to each of several test-tubes of acidified melted agar and immediately poured into petri dishes; (b) by directly transferring small lumps of soil by means of a sterilized platinum loop to the surface of agar in petri dishes. Several media were used, including unacidified potato dextrose agar, acidified potato dextrose agar, soil agar, Jensen's III (35), and an acid-raisin agar recommended by Waksman (85). Acid agars were used in order to keep down bacteria. The fungous colonies, however, frequently outgrew the bacteria on unacidified agar. The plates were incubated at room temperature and at four other temperatures ranging from 7 to 33° C., in order to permit the growth of fungi with different temperature optima.

Soil fungi were isolated indirectly by sowing sterile seedlings on sick soil.[5] This soil was brought from the field in new bags and placed in sterilized pots in the laboratory. The sterile seedlings were then planted and after about three weeks' growth their roots were washed free of soil and examined for disease. Portions of roots showing lesions were then surface sterilized and plated on agar, and fungi were isolated from them. Even with these precautions, it is possible that all the fungi developing on the roots were not from the soil. However, the purpose was to secure fungi pathogenic on the roots rather than to investigate the fungous flora of the soil.

[4] Furnished through the courtesy of W. C. McKillican and S. J. Sigfusson, superintendent and assistant superintendent, respectively, of the Dominion Experimental Farm, at Brandon.

[5] Method of obtaining sterile seedlings is given on page 10.

PURIFICATION OF THE CULTURES

Before inoculation experiments were attempted most of the cultures were purified by subculturing. Obviously this was not possible with several which failed to sporulate. Certain cultures were originally started from single spores. Monosporous cultures were also made from others which proved pathogenic and which were desired for more careful study.

METHOD OF SECURING DISEASE-FREE SEEDLINGS

Disease-free seedlings were necessary for the isolation of fungi indirectly from the soil, as previously stated. They were also required for inoculation studies, and a better method of seed sterilization than that used in the isolation of fungi was needed for pathogenicity tests in the greenhouse. Following the suggestion of Selby and Manns (73), Braun (11), and Norton and Chen (56), it was found that presoaking the seed increased the germicidal efficiency of the disinfectant (Table I). It was also found that in order to obtain uninjured seedlings it was advisable to wash the seeds finally in sterile water, rather than to plate them directly from alcohol. The following method of seed sterilization was therefore used for a time: Presoak in water from six to twelve hours; dip in 70 per cent ethyl alcohol; immerse for from two to five minutes in a 1 to 1000 corrosive sublimate solution, wash in sterile distilled water. The effects of this treatment are not shown in Table I, but they were similar to those shown by No. 4 in this table, except that the seeds were not injured by a final dip in alcohol. It was later found that a silver nitrate treatment essentially similar to that used by Dosdall (19) and based on Schröder's (72) treatment, was more effective, and this method was used subsequently. (See No. 6, Table I.) After sterilization, the treated seeds were plated on potato dextrose agar in petri dishes and allowed to germinate at room temperature, or were thoroly dried for future sowing in the greenhouse. When the plumules of the seedlings were approximately 1 cm. long, those showing no evidence of fungous growth around them were selected as sterile seedlings. While occasionally some of these seedlings did later show evidence of contamination when grown under sterile conditions, this was the exception, especially when bright, plump seeds were selected.

TABLE I

EFFECT OF PRELIMINARY SEED TREATMENT TESTS ON MARQUIS WHEAT

No.	Treatment	No. of seeds treated	Per cent germination	Per cent yielding fungi	Effect on seedlings
1.	Dipped in 70% alcohol; immersed from 2 to 5 minutes in corrosive sublimate solution 1-1000; dipped in 70% alcohol	32	97	81	Injured
2.	Dipped in 70% alcohol; immersed from 2 to 5 minutes in corrosive sublimate solution 1-1000; washed in sterile water	100	100	77	Not injured
3.	Presoaked 12 hours; immersed 5 minutes in alcoholic corrosive sublimate solution (2 gms. per 1000 cc. 70% alcohol); washed once in 70% alcohol; washed thrice in sterile water	125	0	41	Seed killed
4.	Presoaked 12 hours; dipped in 70% alcohol; immersed from 2 to 5 minutes in corrosive sublimate solution 1-1000; dipped in 70% alcohol	80	86	46	Injured
5.	Immersed 12 hours in N/100 silver nitrate solution; washed in sterile sodium chloride solution; dipped in 70% alcohol	46	85	0	Injured
6.	Immersed 7 hours in N/100 silver nitrate solution; washed in sterile sodium chloride solution; washed in sterile water	188	98	23	Not injured

EXPERIMENTAL RESULTS

FUNGI CARRIED BY THE SEED

FUNGI CARRIED BY WHEAT SEEDS IN GENERAL

It would be expected that there would be many fungous spores on the surface of wheat seeds. Bolley (7) found spores of fifteen different genera of fungi in the washings of wheat seed coats in North Dakota, while the following were obtained from seeds treated for one minute with a dilute formalin solution: *Alternaria*, *Colletotrichum*, *Macrosporium*, *Fusarium*, *Cephalothecium*, *Helminthosporium*, and *Penicillium*. He concluded from these results that practically all of the fungi which he and his collaborators had shown to be destructive to the roots of wheat, reached the seed before it was mature.

Hoffer (32) tested the surface sterilized seed of 34 varieties of wheat in Indiana and found 14 free from fungi of any kind, 13 infected with *Fusarium*, 4 with an internal *Macrosporium*, while a *Fusarium* and a *Macrosporium* were isolated from 3. An alcoholic solution of corrosive sublimate was used as a disinfectant.

Wollenweber (89) obtained the following genera from seeds cultured in Germany: *Verticillium*, *Spicaria*, *Alternaria*, *Trichothecium*, *Langloisula*, *Ramularia*, *Melanospora*, *Leptosphaeria*, *Helminthosporium*, *Gibberella*, and *Fusarium* (three species). The mycelium of some was superficial while that of others was internal.

Sherbakoff (71) cultured several samples of wheat seed and concluded that the most common *Fusarium* of wheat in Tennessee, as in other states, was *F. graminearum* Schwabe (*Gibberella saubinetii*) while *Fusarium moniliforme* Sheldon was not common on wheat seed.

Christensen (13) found *Helminthosporium sativum* P. K. and B. prevalent on wheat seeds obtained from widely separated localities.

Stakman (76) found *Alternaria*, *Tilachlidium*, and *Fusarium* on or in wheat seeds, the former being particularly persistent. She (74) also frequently isolated *Helminthosporium*.

The writer found great variation in the fungi on different seed lots. Poor seed, especially samples showing evidence of weathering, immaturity, sprouting, or other defects, was particularly heavily infected; whereas bright, plump, well-matured samples seemed comparatively free from infection. All seeds tested were superficially sterilized as previously described. In general, *Alternaria* was most commonly isolated from wheat grains and apparently is almost universally distributed. *Fusarium graminearum* Schwabe (*Gibberella saubinetii* (Mont.) Sacc.), however, was obtained almost invariably from scabby kernels. Other species of *Fusarium* were frequently isolated, but less commonly than the one named. *Fusarium moniliforme* Sheldon was isolated from seed from widely separated localities, but it was not found commonly. *Helminthosporium sativum* P. K. and B. was usually the most prevalent fungus in black-pointed kernels. It was also frequently isolated from kernels showing no discolorations and is undoubtedly the most common species of *Helminthosporium* on wheat seeds. Another *Helminthosporium*, with narrower spores than those of *H. sativum*, which we shall designate as *Helminthosporium N*, was found several times in cultures of black-pointed seeds. Several small-spored *Helminthosporia*, which we shall consider collectively as *Helminthosporium M*, were obtained in similar cultures. These forms are apparently wide-spread but not particularly prevalent. *Stemphylium*, *Cladosporium*, *Torula*, *Chaetomium*, *Penicillium*, *Aspergillus*, *Epicoccum*, *Cephalosporium*,

Acrostalagmus, Cephalothecium, and *Trichoderma,* as well as several unidentified non-sporulating forms possibly belonging to the *Mycelia Sterilia,* were also isolated from the seed.

FUNGI FOUND IN BLACK-POINTED WHEAT SEEDS

One of the most characteristic discolorations of the seed is "black-point," so named by Bolley because of the conspicuous dark brown or creosote-colored embryos of affected grains. On account of the prevalence of such kernels in apparently good samples of seed wheat, it was considered that these might be important as carriers of root-rotting fungi. Hence an attempt was made to determine the causal organisms and their ability to cause root-rot.

Various investigators have reported the widespread occurrence of black-point and have endeavored to determine its cause. Bolley (7), in 1911 and in 1913 (8), reported the isolation of *Alternaria, Helminthosporium,* and *Fusarium* from black-pointed wheat kernels. He pointed out that *Alternaria* was most commonly associated with black-pointed grains, but stated further that these symptoms might be caused by several fungi. No inoculation experiments were reported, however.

Güssow (27), in 1911, received samples of wheat containing kernels affected with black-point from widely separated regions of Canada (Ontario and Saskatchewan). Germination of these grains did not appear to be impaired, but the seedlings from them were less vigorous than those from normal seeds.

Coons (16) considered that black-point was a disease, or a series of diseases, of little economic importance, which had been present in Michigan for many years. Affected grains germinated well and no distinct lesions formed on the seedlings. He found the diseased tissue swarming with bacteria, which, according to Dr. E. F. Smith, resembled *Bacterium viridi-lividosum.*

Lucia McCulloch (54), in 1920, described a blackening of the embryo ends of wheat kernels from spikelets affected with basal glume rot of wheat. She proved that the disease was caused by a new species of bacterium, *Bacterium atrofaciens* McCulloch.

Stakman (75) reported dark brown blotches on wheat seeds attacked by *Helminthosporium,* but did not mention any particular localization of the discoloration at the germ ends.

The writer (31) inoculated immature wheat heads in various stages of development in the greenhouse at Saskatoon, Sask., with three strains of *Helminthosporium* (tentatively determined as *H. sativum*) from wheat, barley, and rye. Typical black-point developed on numerous kernels in the inoculated heads. Some kernels, especially those from heads inoculated in the early stages of development, showed

brown lesions extending over other parts of the seed as well as the embryo, but usually the discolorations were most pronounced at the germ end. The typical fungus was later recovered from the diseased kernels.

Nevada S. Evans (24) isolated a *Helminthosporium* similar to *H. sativum*, in most instances from black-pointed durum wheat kernels. Artificial inoculations were made in the field on the durum wheat varieties Acme and Pentad, in the flowering stage. Many black-pointed kernels developed in the inoculated heads in contrast to a low percentage in the controls.

Christensen (13) noted that the germ ends of wheat seeds were most conspicuously discolored by *H. sativum*.

Weniger (86) stated that black-pointed kernels of durum wheat in North Dakota, tho sometimes shriveled, were usually normal in weight but frequently germinated poorly and produced blighted seedlings.

Drechsler (20) reported low viability of wheat seeds discolored by *H. sativum*. He also noted that a lighter brownish discoloration of the seeds was associated with *Alternaria* and other fungi.

The writer found black-pointed kernels frequently in samples of common wheat from different parts of the United States and Canada and in two samples from the Argentine. A sample of Red Bobs wheat from Western Canada contained 34 per cent of these kernels. A lot of Marquis wheat grown at St. Paul in 1920, and containing 13.4 per cent black-pointed kernels, was used in the following cultural studies.

Two hundred normal seeds and two hundred black-pointed seeds were selected from this sample, presoaked for seven hours, surface sterilized in corrosive sublimate solution, 1 to 1000, washed in sterile distilled water, and plated on potato dextrose agar in petri dishes. The cultures were incubated at room temperature until the colonies of fungi growing out from the seeds were sporulating abundantly. They were then examined, and counts were made of the different fungi which had developed. The results of this test are recorded in Table II.

When the seeds germinated, some were raised off the agar by the growth of the young seedlings before any fungi grew out from them. The percentage of total seeds yielding fungi is therefore, no doubt, lower that it would have been had this not occurred. It is quite evident that *H. sativum* was the most common fungus associated with black-point in this sample and that it was much more common on diseased kernels than on normal kernels. It is also significant that two other apparently distinct species of *Helminthosporium* were obtained from the black-pointed kernels and not from normal ones.

TABLE II

COMPARISON OF THE FUNGOUS CONTENT OF BLACK-POINTED AND NORMAL MARQUIS WHEAT SEEDS FROM THE SAME SAMPLE

Fungi developing	Black-pointed seeds			Normal seeds		
	No. seeds plated on agar	Per cent of total seeds plated	Per cent of seeds yielding fungi	No. seeds plated on agar	Per cent of total seeds plated	Per cent of seeds yielding fungi
Helminthosporium sativum	97	48.5	73.5	4	2.0	33.3
Helminthosporium N	5	2.5	3.8	0	0.0	0.0
Helminthosporium M	5	2.5	3.8	0	0.0	0.0
Alternaria	14	7.0	10.6	5	2.5	41.7
Other fungi	11	5.5	8.3	3	1.5	25.0
None	68	34.0	...	188	94.0	...

A comparison also was made of the relative value for seed purposes of black-pointed kernels and normal kernels from the same sample of Marquis wheat. These tests were made both in the field and in the greenhouse. In the greenhouse, plantings were made on December 31, 1921, on both steam-sterilized and unsterilized soil, and notes were taken one month later (see Table III).

TABLE III

COMPARISON OF GERMINATION AND SEEDLING DEVELOPMENT OF BLACK-POINTED AND NORMAL MARQUIS WHEAT KERNELS IN THE GREENHOUSE

Soil treatment	Kind of seed	No. of seeds planted	No. of seeds which germinated	Per cent of seeds which germinated	Mean height of plants, cm.
Sterilized soil	Normal	100	100	100	21.47±.10
	Black-pointed	100	89	89	19.54±.30
Unsterilized soil	Normal	400	356	89	21.23±.14
	Black-pointed	400	336	84	21.17±.17

It should be noted that only plump black-pointed kernels were used in these experiments, so that any differences can be attributed more directly to the effect of the organisms carried by such seeds than if shriveled kernels were compared with normal ones. It is evident that the viability of the seed is reduced somewhat by the disease, and on the sterilized soil there was a significant difference in growth, as measured by mean height of tops, but there was no significant difference on unsterilized soil. No particular difference was noted in the amount of root- and foot-rots on the plants in the different series, so it would seem that conditions were not particularly favorable for the development of the fungi in this experiment.

The sample of Marquis wheat used in the field tests germinated rather poorly. Sixty-seven per cent of the apparently normal kernels germinated, while only 50 per cent of the black-pointed ones were viable. Rod rows of one hundred seeds each were planted at two

different dates and the plants allowed to develop to maturity. Only 30 per cent of the black-pointed seeds germinated in the first planting as compared with 61 per cent of the normal seeds. Consequently the differences in yield were large. Much less marked differences, however, occurred in the second planting, so it would appear that environmental conditions play an important part in determining the degree of damage resulting from the use of black-pointed kernels for seed. (See Table IV.)

TABLE IV

Effect of Black-Point on Germination and Yield of Marquis Wheat in the Field

Date sown	Kind of seed	No. of seeds planted	Per cent germination	Total weight of mature plants, gms.	Total weight of grain, gms.
May 20	Normal	100	61	363.0	64.5
	Black-pointed	100	30	142.0	25.5
May 25	Normal	100	60	148.0	18.0
	Black-pointed	100	51	146.5	17.5

In order to ascertain whether fungi other than *H. sativum,* which were found associated with black-pointed kernels, might not also be responsible for embryo discolorations, inoculations were made on immature wheat heads in the greenhouse and in the field, with several fungi isolated from wheat seeds. As it seemed probable that *Fusaria* and other light-colored species were less likely to cause creosote discolorations, only dematiaceous forms were chosen for these studies.

The cultures used sporulated well on potato dextrose agar and were grown on this medium to obtain inoculum. Wheat heads were inoculated by transferring spores and mycelium from the surface of agar slants to the outer surface of the wheat spikelets. Heads were selected in which the grain was mostly in the milk stage, altho heads in flower and those with only partly formed grain were also inoculated. They were atomized with tap water before and after inoculation. Only the well-developed central spikelets of the heads were inoculated, and counts were made later of only the two outer grains in each spikelet, that is, the largest and best developed seeds. Controls were treated in a similar manner except that no inoculum was applied to them. In the greenhouse experiments, the inoculated plants and controls were placed in moist incubation chambers for forty-eight hours after inoculation and were then placed on greenhouse benches. In the field, the inoculations were made an hour or two before sundown, and the heads were then covered with glassine bags for forty-eight hours.

Table V summarizes the results of greenhouse inoculations made on May 28 and August 25, 1922, and field inoculations made on July 8.

TABLE V

RELATIVE ABILITY OF DIFFERENT FUNGI ISOLATED FROM WHEAT SEEDS TO CAUSE BLACK-POINT OF MARQUIS WHEAT

Fungi	Source of seed from which fungi were obtained	No. of inoculated seeds examined		No. of inoculated seeds which developed black-point		Per cent of inoculated seeds which developed black-point	
		Greenhouse	Field	Greenhouse	Field	Greenhouse	Field
Helminthosporium sativum	Buffalo, Okla.	43	39	23	8	53.5	20.5
Helminthosporium N	St. Paul, Minn.	26	59	11	8	42.3	13.6
Helminthosporium M	St. Paul, Minn.	67	56	16	5	23.9	8.9
Stemphylium parasiticum	Eureka, Calif.	43	75	10	7	23.3	9.3
Stemphylium sp.	Unknown	22	28	0	0	0	0
Torula sp.	Indian Head, Sask.	15	65	0	1	0	1.5
Alternaria sp.	Boerne, Texas	46	83	0	1	0	1.2
Cladosporium sp.	Beaver Lodge, Alta.	15	26	1	0	0	0
Control		59	78	0	0	0	0

As indicated in Table V, four cultures, representing at least two genera and four species, caused black-point both in the greenhouse and in the field. The pathogenicity of each was proved by following out the rules of proof. All three cultures of *Helminthosporium* attacked the glumes as well as the seed. *Helminthosporium N* and *Helminthosporium M* each produced a brown discoloration of the glumes very similar to that caused by *H. sativum* and both were recovered therefrom. Each also caused floret sterility when heads in the flowering stage were inoculated. Their relative virulence as root-rotting organisms will be discussed later. While these results again indicate that *H. sativum* probably is the principal cause of black-point, they also show that other species (31) can cause it (Plate II, Fig. 1). *Stemphylium parasiticum* (Thüm.) Elliot is probably unimportant, as it was isolated only once by the writer. The positive results with *Torula* and *Alternaria* in the field were inconclusive, as only one black-pointed kernel developed in either case, and neither fungus was recovered. The fact that greenhouse inoculations were unsuccessful also indicates that these cultures are nonpathogenic. Two other strains of Alternaria were also tried but with the same results. *Stemphylium parasiticum* was isolated from the one black-pointed kernel which developed in a head inoculated with *Cladosporium*, so there evidently had been accidental contamination with that fungus.

The fact that a lower percentage of successful infections was obtained in the field than in the greenhouse can well be attributed to the dry weather and consequent unfavorable conditions for infection.

SOIL FUNGI

Waksman (85) gives a good historical review and an extensive bibliography of the work on soil fungi previous to 1916. In an examination of eight different soils, more than 100 distinct species of fungi belonging to 31 genera, were isolated. The genera most commonly found were *Penicillium, Mucor, Aspergillus, Trichoderma, Cladosporium, Fusarium, Cephalosporium, Rhizopus, Zygorhynchus, Acrostalagmus, Alternaria,* and *Verticillium.* It is pointed out that Köning found 7 of these 12 genera; Dale, 10; Jensen, 9; Goddard, 9; and McLean and Wilson, 8, indicating their general distribution in the soil. *Mucor, Penicillium, Aspergillus,* and *Trichoderma* were found by all six investigators (including Waksman), showing that these are probably common in most soils. It is stated that more than 50 genera of fungi have been found in the soil and that the largest numbers occur within the upper four inches.

Beckwith's (3) work is of particular interest here, as he investigated the fungous flora of wheat-sick soils. The following genera were found in the soil of a plot on the North Dakota Agricultural Experiment Station farm, which had been cropped to wheat continuously for forty years: *Colletotrichum* (two species), *Macrosporium, Alternaria, Spicaria, Verticillium, Rhaphalomyces, Cephalothecium,* and *Helminthosporium.* Five of these, namely, *Fusarium, Colletotrichum, Macrosporium, Alternaria,* and *Helminthosporium,* are said to be pathogenic to wheat. These fungi were seldom found in virgin soil. It is considered, therefore, that this is additional evidence that part of the deterioration in wheat yields of the Northwest may be due to soil fungi.

Bolley (7) presents the results of another series of cultures made by Beckwith from the soil of some wheat fertilizer plots at the North Dakota Experiment Station. These were made at intervals from February to July, 1911. In February *Alternaria, Colletotrichum, Fusarium,* and *Verticillium* were isolated, while in June only the first three were obtained. It is interesting to note that *Helminthosporium* was not obtained from these soils.

In the isolations made by the writer, *Helminthosporium* was never obtained directly from the soil. It was isolated, however, from the roots of sterile seedlings sown on sick soil. Several species of *Fusarium* were found rather frequently in the soil, but only a few were identified. *Fusarium moniliforme* Sheldon was obtained directly from the soil of a continuous wheat plot at University Farm, St. Paul, and also from a soil secured from Brandon, Manitoba. It also was isolated from the roots of sterile wheat seedlings planted on sick soil. *Fusarium betae* (Desm.) Sacc. (identification by Dr. Sherbakoff) was also obtained from the Brandon soil. A *Fusarium* species, placed by Dr. Sherbakoff

in the section Elegans but not determined specifically, was isolated from the roots of sterile seedlings grown on a continuous wheat plot at St. Paul. Green species of *Trichoderma* occurred quite commonly in cultures from the soil, and a species of *Phoma* was obtained several times from soil at University Farm. *Colletotrichum* was not isolated from any of the soils used, altho from Beckwith's results it was expected. Species of *Penicillium, Aspergillus,* and *Mucor* were rather common. Species of *Rhizopus, Alternaria, Cladosporium, Chaetomium, Cephalosporium, Stilbella, Acrostalagmus,* several non-sporulating forms, some which produced only chlamydospores, and several which were not identified, also were found. As the principal object was to find pathogenic forms, time did not allow a careful study of many interesting non-pathogenic species.

FUNGI FROM MISCELLANEOUS SOURCES

Several fungi were isolated from root and foot lesions of wheat and other cereals, and some were obtained from other sources. Many of the same species and genera obtained from the seed and from the soil are also represented here. A few different ones, however, were secured. *Colletotrichum graminicolum* (Ces.) Wilson was isolated from the foot of a mature wheat plant. *Pleospora sp.*, probably *P. trichostoma* (Fr.) Wint., which proved to be the perfect stage of an *Alternaria*, was isolated from old wheat straw. *Helminthosporium gramineum* (Rab.) Erik. was obtained from a single spore from a typical stripe lesion on a barley leaf, while a culture of *Helminthosporium teres* Sacc. was obtained in a similar manner from a typical net-blotch lesion on barley, and a species of *Helminthosporium* apparently differing from any previously described species was obtained from a diseased wheat root. *Piricularia grisea* (Cke.) Sacc. was isolated from a leaf-spot on a seedling millet leaf. In addition, several cultures were obtained from other workers for which grateful acknowledgment is herewith made, e.g., *Helminthosporium sp.* (of the general *H. sativum* type) from J. J. Christensen (originally from Dr. F. L. Stevens); herbarium cultures of *Fusarium culmorum* (W.G.Sm.) Sacc. and *Fusarium lini* Bolley from Dr. Louise Dosdall; and a *Fusarium moniliforme* Sheldon culture from Dr. W. D. Valleau.

PRELIMINARY PATHOGENICITY STUDIES

In order to obtain a preliminary indication of the parasitism of the different cultures, essentially the same method as that used by Stakman (76) was employed. Sterile Marquis wheat seedlings were prepared as previously described and planted in tubes of nutrient agar. Modified Sach's solution or the three-salt solution of Livingston and Tottingham,

R_8C_1, (47) plus 2 per cent agar, proved equally satisfactory. Equal quantities of this medium were placed in each of several large test tubes, and these were then plugged and sterilized. A 1 by 8 inch or a 1 by 12 inch tube was usually used, but occasionally smaller ones were employed. The medium was tubed when the seedlings were ready to be transferred, so that the agar would still be comparatively soft. A single seedling was planted in each tube by means of sterile forceps. The seedlings were then arranged with a sterile platinum needle so that the rootlets penetrated into the soft agar. They were then inoculated with different fungi. This was accomplished by placing a bit of mycelium from the surface of an agar slant of each culture on the surface of the agar at the junction of the seed and roots. Several tubes of each series were left uninoculated. The tubes were inserted in jars of sand so that the roots were in comparative darkness, and the jars were then placed near a window in the laboratory. The final notes were usually taken after about three weeks. The degree of parasitism of the different fungi was then judged by their effect on the development of the seedlings, but particular attention was given to evidences of root parasitism as judged by stunting, deformity, discoloration, and other conditions of the roots as compared with the white uninjured roots of the controls. The results of these preliminary tests are summarized in Table VI (see also Plate I).

On the basis of these trials it was possible to reject many cultures, so far as further pathogenicity tests were concerned. It was considered that any cultures marked "—" did not merit further trial except for comparative purposes. It was also considered that most of the weak pathogenes could be discarded without danger of eliminating any which would be likely to prove destructive in the field. The fact that such well known pathogenes as *Gibberella saubinetii* and *Helminthosporium sativum* always parasitized the plants, whereas such saprophytes as *Cladosporium*, *Cephalothecium*, and *Chaetomium* did not, indicates that these tests at least gave a rough indication of the relative pathogenicity of the different fungi.

TABLE VI

SUMMARY OF RESULTS OF INOCULATING ROOTS OF MARQUIS WHEAT SEEDLINGS GROWING IN AGAR, WITH DIFFERENT FUNGI

Fungus	Source	No. of cultures tested	Degree of parasitism*		
			+	±	—
Alternaria spp.	Seed	47	6	35	6
Stemphylium parasiticum	"	1	0	1	0
Stemphylium sp.	"	1	0	0	1
Helminthosporium sativum	"	11	11	0	0
Helminthosporium N	"	3	2	1	0
Helminthosporium M	"	3	1	2	0
Torula sp.	"	2	0	0	2
Cladosporium sp.	"	2	0	0	2
Gibberella saubinetii	"	3	3	0	0

TABLE VI—*Continued*

Fungus	Source	No. of cultures tested	Degree of parasitism*		
			+	±	−
Fusarium moniiforme	Seed	4	2	2	0
Fusarium spp.	"	8	4	4	0
Cephalothecium roseum	"	2	0	0	2
Aspergillus spp.	"	6	1	2	3
Penicillium spp.	"	4	0	1	3
Acrostalagmus spp.	"	3	0	2	1
Chaetomium sp.	"	1	0	0	1
Trichoderma sp.	"	1	0	1	0
Epicoccum sp.	"	2	0	0	2
Other fungi	"	15	0	9	6
Fusarium moniliforme	Soil (direct)	2	2	0	0
Fusarium betae	" "	1	1	0	0
Fusarium spp.	" "	15	10	4	1
Trichoderma spp.	" "	7	3	4	0
Phoma sp.	" "	2	1	1	0
Acrostalagmus sp.	" "	1	0	1	0
Cephalosporium sp.	" "	2	0	2	0
Stilbella sp.	" "	1	0	0	1
Rhizopus spp.	" "	8	0	0	8
Mucor spp.	" "	5	0	0	5
Penicillium spp.	" "	7	0	3	4
Aspergillus spp.	" "	9	0	4	5
Cladosporium sp.	" "	3	0	0	3
Alternaria sp.	" "	2	0	2	0
Chaetomium sp.	" "	2	0	0	2
Other fungi	" "	26	0	20	6
Helminthosporium sativum	Soil (on roots)	1	1	0	0
Fusarium moniliforme	" " "	1	1	0	0
Fusarium spp.	" " "	6	4	1	1
Fusarium sp. (Elegans section)	" " "	1	1	0	0
Rhizopus sp.	" " "	1	0	0	1
Cephalosporium sp.	" " "	1	0	1	0
Other fungi	" " "	4	0	0	4
Helminthosporium sativum	Wheat (foot and roots)	6	6	0	0
Helminthosporium pedicellatum n.sp.	Wheat (roots)	1	0	1	0
Fusarium sp. (Elegans section)	" "	1	1	0	0
Fusarium spp.	" "	5	3	2	0
Gibberella saubinetii	" "	3	3	0	0
Colletotrichum graminicolum	" "	1	1	0	0
Acrostalagmus sp.	" "	3	1	2	0
Aspergillus sp.	" "	1	0	0	1
Trichoderma sp.	" "	1	0	1	0
Cephalosporium sp.	" "	1	0	1	0
Chaetomium sp.	" "	1	0	0	1
Pleospora sp.	" "	1	0	1	0
Penicillium sp.	" "	1	0	0	1
Rhizoctonia sp.	" "	2	2	0	0
Other fungi	" "	22	0	15	7
Fusarium lini	(herbarium)	1	1	0	0
Fusarium culmorum	"	1	0	1	0
Helminthosporium sativum	(Stevens)	1	1	0	0
Gibberella saubinetii	(rye)	1	1	0	0
Fusarium spp	(miscellaneous)	4	2	1	1
Piricularia grisea	(millet)	1	0	0	1
Other fungi	(miscellaneous)	15	0	8	7
Totals		301	76	136	89

* + Parasitic. ± Weakly parasitic. − Non-parasitic.

PATHOGENICITY TESTS IN THE GREENHOUSE

The selected cultures were now given a thoro test in inoculated soil in the greenhouse. Each fungus was tested by inoculating the soil in four 4-inch pots with a pure culture, and planting each with twenty-five seeds of Marquis wheat, so that each series consisted of a test of one hundred seeds. The seeds were surface sterilized as previously described. The soil used was a mixture of two parts garden loam and one part sand. This was steamed usually for three hours on each of two successive days. The inoculum was grown in small Erlenmeyer flasks, on sterilized wheat seed soaked with water. All the fungi grew well on this medium and it was afterwards easily broken up and distributed through the soil. As nearly an equal quantity as possible of inoculum was added to each of the pots. The controls were treated in a similar manner except that the sterilized uninoculated medium was introduced into the soil instead of the inoculated medium. The different pots of the different series were placed some distance apart on greenhouse benches in order to prevent the spread of inoculum from one series to another. Notes were taken after about thirty days. The contents of the pots were removed and the soil was washed away from the roots in running water. Notes were then taken on both roots and tops. The number of seedlings that survived was recorded, and the number of these which showed root- or foot-rot and the severity of each was noted. Relative growth was measured by taking the total dry weight of the tops and in some instances their mean height. The former was considered the more reliable, however, and was used throughout, while the latter was frequently omitted in order to reduce the amount of work. These tests were continued from July 5, 1922, to March 11, 1923, and the results are presented in Table VII. Plate III, Figure 2, shows a typical control series compared with one in which *Fusarium moniliforme* was used as the inoculum.

TABLE VII

RESULTS OF INOCULATING MARQUIS WHEAT WITH DIFFERENT FUNGI

Series No.	Fungus	Length of test in days	Source of culture*	Stunted seedlings†‡	Seedlings with root-rot†‡	Seedlings with foot-rot†‡	Mean height, cms.	Total dry weight of tops, gms.	Observations
		7/5-8/5							
1	Fusarium moniliforme	31	Seed	.....	$57^{h}/57$	$55^{l}/57$	8.8	0.76	10 plants dead
2	Fusarium sp.	"	"	.....	$69^{l}/73$	$44^{l}/73$	41.9	6.45	
3	Helminthosporium gramineum	"	Barley leaf	.....	$38^{l}/76$	$1^{tr}/76$	41.7	6.89	
4	Unknown (formed chlamydospores only)	"	Soil	.....	$42^{l}/66$	$3^{tr}/60$	40.7	6.12	
	Control	"		.....	$36^{l}/68$	$4^{tr}/68$	38.0	5.75	
		7/5-8/7							
5	Trichoderma sp.	33	"	.....	$71^{l}/74$	$4^{tr}/74$	37.7	5.24	
6	Fusarium sp.	"	Seed	.....	$7^{tr}/75$	0/75	41.1	8.09	
7	Fusarium sp.	"	Soil	.....	$40^{l}/74$	0/74	42.2	6.71	
8	Fusarium sp.	"	"	.....	$38^{l}/65$	0/65	45.5	6.40	
9	Unknown (formed chlamydospores only)	"	"	.....	$3^{l}/70$	0/70	38.4	7.38	
	Control	"		.....	$22^{tr}/56$	0/56	35.8	5.74	
		7/25-8/25							
10	Phoma sp.	31	Soil	1/62	$39^{l}/62$	$15^{tr}/62$	...	6.17	
11	Helminthosporium sativum	"	Roots	7/53	$53^{m}/53$	$20^{l}/53$	...	4.42	
12	" "	"	Seed	9/62	$62^{m}/62$	$30^{l}/62$	...	6.29	
13	Helminthosporium M. Strain 1	"	"	7/58	$57^{m}/58$	0/58	...	6.64	
14	Helminthosporium N	"	"	5/37	$37^{m}/37$	$3^{tr}/37$	...	4.14	
15	Helminthosporium sativum	"	Furnished by F. L. Stevens	8/48	$48^{m}/48$	$6^{m}/48$	...	5.92	
	Control	"		7/49	$21^{tr}/49$	$3^{tr}/49$	...	4.59	
		7/31-8/31							
16	Gibberella saubinetii	31	Seed	3/3	$2^{h}/3$	$2^{tr}/3$	6.0	0.01	2 plants dead
17	Helminthosporium sativum	"	"	8/36	$36^{h}/36$	.....	37.3	3.59	1 plant dead
18	Trichoderma sp.	"	Soil	2/51	$13^{tr}/51$	$2^{tr}/51$	...	6.65	
19	Alternaria sp.	"	Seed	3/54	$11^{tr}/54$	$3^{tr}/54$	...	7.57	
20	Rhizoctonia	"	Roots	1/52	$20^{tr}/52$	$5^{tr}/52$	...	7.13	
	Control	"		7/79	$14^{tr}/79$	0/79	40.4	7.53	

TABLE VII—*Continued*

RESULTS OF INOCULATING MARQUIS WHEAT WITH DIFFERENT FUNGI

Series No.	Fungus	Length of test in days	Source of culture*	Stunted seedlings†‡	Seedlings with root-rot†‡	Seedlings with foot-rot†‡	Mean height, cms.	Total dry weight of tops, gms.	Observations
		7/29 9/1							
21	Trichoderma sp.	34	Seed	4/55	30tr/55	11tr/55	...	6.83	
22	Helminthosporium teres	"	Barley leaf	4/67	7tr/67	2tr/67	...	9.74	
23	Aspergillus niger	"	Seed	5/52	31l/52	1tr/52	...	5.98	
24	Unknown (formed chlamydospores only)	"	Soil	2/42	10tr/42	2tr/42	...	5.65	
25	Fusarium sp.	"	Roots	4/56	24l/56	0tr/56	...	6.29	
26	Unknown (formed chlamydospores only)	"	Soil	3/55	9tr/55	0/55	...	5.63	
27	Fusarium sp.	"	"	7/51	27l/51	9tr/51	...	5.00	
	Control	"		6/76	11tr/76	0/76	40.7	8.21	
		7/31-9/5							
28	Gibberella saubinetii	36	Roots	6/28	27l/28	0/28	41.9	3.32	
29	Fusarium sp. (Elegans section)	"	Soil (on roots)	1/6	6m/6	0/6	41.4	0.49	
30	Gibberella saubinetii	"	Roots	6/34	30m/34	8l/34	36.7	1.22	1 plant dead
31	Fusarium lini	"	Herbarium	6/49	42l/49	0/49	37.5	4.74	
32	Fusarium sp.	"	Roots	1/47	15l/47	0/47	40.5	4.39	
33	Fusarium sp.	"	Barley foot	2/63	63tr/63	0/63	34.7	4.48	
34	Fusarium sp.	"	Soil	4/60	8tr/60	0/60	...	6.72	
35	Fusarium sp.	"	Roots	4/46	29tr/46	1tr/46	...	4.34	
36	Fusarium sp.	"	Soil	3/59	26tr/59	0/59	...	5.53	
	Control	"		4/67	5tr/67	0/67	42.4	6.48	
		8/11-9/12							
37	Helminthosporium M. Strain IV	32	Millet leaf	2/20	20m/20	0/20	28.1	1.13	
38	Phoma sp.	"	Soil	0/40	31l/40	2tr/40	37.7	3.69	
39	Alternaria sp.	"	Seed	3/53	14l/53	0/53	...	4.07	
40	Fusarium moniliforme	"	"	13/25	25h/25	6l/25	24.1	1.16	2 plants dead
41	Fusarium sp.	"	Foot	15/72	51l/72	0/72	29.0	3.69	
42	Alternaria sp.	"	Seed	4/79	17tr/79	0/79	...	5.07	
43	Alternaria sp.	"	Seed	3/72	16tr/72	1tr/72	...	5.41	
44	Alternaria sp.	"	"	1/77	31l/77	3tr/77	...	4.79	
	Control	"		3/64	3tr/64	0/64	36.7	5.41	

TABLE VII *Continued*

RESULTS OF INOCULATING MARQUIS WHEAT WITH DIFFERENT FUNGI

Series No	Fungus	Length of test in days	Source of culture*	Stunted seedlings†‡	Seedlings with root-rot†‡	Seedlings with foot-rot†‡	Mean height, cms.	Total dry weight of tops, gms.	Observations
		8/17-9/18							
45	Fusarium sp. (Elegans section)	32	Foot	5/18	$12^{l}/18$	0/18	23.7	0.57	
46	Fusarium moniliforme	"	Soil	50/60	$60^{h}/60$	$2^{tr}/60$	20.3	0.90	6 plants dead
47	Fusarium moniliforme	"	Seed	13/31	$31^{m}/31$	$1^{tr}/31$	23.1	1.07	1 plant dead
48	Acrostalagmus sp.	"	Roots.	9/77	$55^{l}/77$	0/77	...	3.91	
49	Stemphylium parasiticum	"	Seed	8/77	$31^{tr}/77$	2/77	...	5.94	
50	Pink fungus (no spores)	"	Roots	4/35	$23^{l}/35$	$7^{tr}/35$	35.1	2.62	2 plants dead
	Control	"		4/62	$10^{tr}/62$	$2^{tr}/62$	34.2	4.25	
		8/28-9/30							
51	Chaetomium sp.	33	Seed	3/92	$13^{l}/92$	0/92	...	3.44	
52	Fusarium sp.	"	"	5/84	$8^{l}/84$	0/84	...	3.64	
53	Gibberella saubinetii	"	Seed	4/31	$24^{m}/31$	0/31	24.3	1.19	7 plants dead
54	Alternaria	"	"	7/75	$33^{l}/75$	0/75	...	2.49	
55	Helminthosporium sativum	"	Roots	3/58	$52^{l}/58$	29/58	...	3.66	
56	Gibberella saubinetii	"	Seed	6/15	$12^{m}/15$	$8^{m}/15$	16.8	0.33	3 plants dead
57	Gibberella saubinetii	"	Roots	17/23	$23^{m}/23$	$16^{l}/23$	13.9	0.50	13 plants dead
	Control	"		8/85	$19^{tr}/85$	0/85	29.7	3.68	
		9/12-10/15							
58	Trichoderma	33	Soil	3/85	$27^{tr}/85$	0/85	...	4.14	1 plant dead
59	Acrostalagmus	"	Roots	13/88	$36^{l}/88$	0/88	...	3.69	" " "
60	Colletotrichum graminicolum	"	Foot	6/84	$45^{l}/84$	1/84	...	4.16	
61	Fusarium sp.	"	Rye foot	17/62	$41^{l}/62$	0/62	...	2.31	" " "
62	Fusarium sp.	"	Soil	3/90	$7^{tr}/90$	0/90	...	5.20	
63	Fusarium sp.	"	Roots	8/80	$8^{tr}/80$	$3^{tr}/80$	...	4.30	
	Control	"		11/74	$26^{l}/74$	0/74	...	3.21	
		10/14-11/14							
64	Helminthosporium M. Strain 1	31	Seed	60/68	$68^{m}/68$	$36^{l}/68$	9.5	0.44	Tops attacked by mice
65	Helminthosporium M. Strain IV	"	Millet leaf	8/84	$84^{l}/84$	$10^{l}/84$	...	...	Tops attacked by mice

TABLE VII—*Continued*

RESULTS OF INOCULATING MARQUIS WHEAT WITH DIFFERENT FUNGI

Series No.	Fungus	Length of test in days	Source of culture*	Stunted seedlings†‡	Seedlings with root-rot†‡	Seedlings with foot rot†‡	Mean height, cms.	Total dry weight of tops, gms.	Observations
66	Helminthosporium M. Strain II	31	Seed	4/76	76^{tr}/76	0/76	...	...	Tops attacked by mice
67	Helminthosporium M. Strain III	"	"	30/94	56^{tr}/94	0/94	...	1.94	Tops attacked by mice
68	Helminthosporium pedicellatum n. sp.	"	Roots	8/72	42^{tr}/72	2^{tr}/72	...	1.74	Tops attacked by mice
69	Cephalosporium sp.	"	"	10/84	26^{tr}/84	0^{tr}/84	...	1.96	Tops attacked by mice
70	Cephalosporium sp.	"	Soil	12/64	8^{tr}/64	0/64	...	1.50	Tops attacked by mice
	Control	"		16/72	28^{tr}/72	0/72	26.5	1.60	Tops attacked by mice
		1/5-2/12							
71	Acrostalagmus sp.	38	Soil	17/90	65^{l}/90	19^{tr}/90	25.1	3.72	
72	Fusarium sp.	"	"	3/93	93^{tr}/93	9^{tr}/93	31.0	5.98	
73	Acrostalagmus sp.	"	Roots	31/92	92^{m}/92	20^{tr}/92	22.2	3.80	
74	Fusarium sp.	"	Soil	8/88	23^{l}/88	5^{tr}/88	29.3	5.44	
75	Fusarium sp.	"	Roots	6/76	10^{tr}/76	3^{tr}/76	25.7	...	
	Control	"		11/91	0/91	0/91	27.8	5.03	
		1/27-3/11							
76	Cephalosporium sp.	43	Soil	7/97	32^{tr}/97	0/97	33.0	9.03	
77	Fusarium sp.	"	"	0/94	13^{tr}/94	0/94	37.4	9.06	
78	Fusarium betae	"	"	6/98	34^{tr}/98	0/98	31.9	7.34	
	Control	"		1/97	0/97	0/97	32.5	6.84	

* Plant part sources refer to wheat where not otherwise specified.

† The denominator of the fractions represents the total number of seeds which produced plants. As 100 seeds were sown in each series, the number also indicates the percentage of seeds from which plants developed. The numerator represents the number of diseased plants, including those which were stunted or affected with root- or foot-rots.

‡ ^{h} = heavy. ^{m} = moderately heavy. ^{l} = light. ^{tr} = trace.

As an abundance of inoculum was used in these tests, it would seem that any virulent pathogenes would have a pronounced effect on the growth of the plants. None, however, except certain species of *Fusarium* and *Helminthosporium* was very virulent. Several other fungi did attack the roots, but in general not severely enough to interfere seriously with the growth of the plants. It scarcely seems probable that they would do much damage in the field, where the inoculum would be less plentiful. Several cultures which were marked "+" in the preliminary tests were either only slightly pathogenic or non-pathogenic in the greenhouse, e. g., numerous species of *Fusarium*. It will be noted that the control plants usually developed some root-rot also. This apparently was because the medium added to the soil formed a suitable substratum for the growth of any fungi which happened to be washed down from the surface of the soil. In the inoculated pots, on the other hand, the medium from the beginning of the experiment would be thoroly permeated with the mycelium of one fungus and hence others would not readily grow there.

DISCUSSION OF DIFFERENT FUNGI AND THEIR EFFECT ON THE HOST

FUSARIUM SPP.

Fusarium graminearum, Schwabe (*Gibberella saubinetii* (Mont.) Sacc.).—This is a well-known parasite of wheat and other cereals (73, 1, 2, 36, 25, 53). It was the most virulent of any of the fungi studied. In most of the trials with this organism in the greenhouse, all or nearly all the seedlings were killed before they emerged from the soil. The virulence of different cultures varied somewhat. Two cultures, one isolated from wheat seed obtained from the Argentine, and another from seed from North Dakota, were particularly destructive. (Table VII—series 56 and 16.) The roots of surviving seedlings were usually stunted and badly rotted, altho occasionally one would develop a few normal roots and apparently recover. The affected roots were usually light brown to reddish brown in color. In the roots examined, the central cylinder usually was destroyed first and the cortex later. The mycelium was intracellular in the stele. All stages of decay were noted, from a slight browning of a few cell walls to a general disintegration. Some of the cells were frequently completely filled with a brown mass of material which probably consisted partly of mycelium and partly of disintegration products of the decaying cell walls. In the cortex the mycelium was both intra- and intercellular. There is little doubt that the importance of this fungus in the field as a cause of seedling blight is in large measure due to its destructiveness on the roots.

Fusarium culmorum (W. G. Sm.) Sacc.—While this fungus is frequently confused with *Fusarium graminearum* Schwabe, it is undoubtedly a distinct species (89). Its ability to cause root-rot of wheat was carefully studied by Stakman (76), who found it very destructive on all parts of the roots. She observed that the vascular tissue was usually attacked first, after which the hyphae spread out through the cortex. In the stele the hyphae were found inside the cells, whereas in the cortex they were mostly intercellular. The writer did not carefully study the relation of this organism to the host. A herbarium culture of the fungus was used in preliminary pathogenicity tests but it was only weakly parasitic and hence was not studied further. Possibly the virulence of the culture had been diminished by long-continued growth on artificial media.

Fusarium moniliforme Sheldon.—Sheldon (69) first found *F. moniliforme* in 1903 on corn in Nebraska, and described it as a new species. A similar fungus found on corn was described by Saccardo (66) as *Oospora verticilloides* Sacc. This in all probability was *F. moniliforme*, but Saccardo failed to observe the macroconidia. Chen (12) isolated a fungus corresponding to Saccardo's *Oospora verticilloides*, but states that if macroconidia were found it would be necessary to describe it as *F. moniliforme* Sheldon. During the last few years, the relation of *F. moniliforme* to foot- and root- and kernel rots of corn has been studied rather extensively in this country. Valleau (84) summarizes the literature on this subject up to 1920. Manns and Adams (48, 49) more recently present further data on its distribution, importance, and prevalence. In a later paper (50) they discuss this fungus as an internal parasite of seed corn. The only previous mention of the occurrence of *F. moniliforme* on wheat that has come to the writer's attention is that of Sherbakoff (71) who states that he found it on wheat seed, altho not often.

As has been mentioned, the writer isolated *Fusarium moniliforme* from wheat seed, wheat roots, and wheat-sick soil. The identity of the fungus, however, was not determined until after it had been tested in the greenhouse for the first time (Table VII, Series 1). The results of that test were so pronounced that an attempt was immediately made to identify the fungus.

It was not readily identified at first, because the culture examined was growing on potato dextrose agar, and on that medium macroconidia were rare. Both macroconidia and microconidia were abundant on the sterilized wheat seed medium, however. These agreed in all essential characters with Sheldon's description and figures of *F. moniliforme*. In order to prove, however, that the two spore forms belonged to the same fungus, six single spore cultures were started

from microconidia, and six from macroconidia. In every instance both types of conidia were produced by the single spore cultures when grown on sterilized wheat seed. In subsequent work only single spore cultures were used.

It was thought that strains of this fungus from different sources might be different. Valleau (84), for instance, found at least two and possibly three distinct strains on corn. Sherbakoff (71) also observed differences in different cultures answering the general description of this species, and proposed a new section of the genus *Fusarium,* namely, Moniliform, to include those forms producing their microconidia in chains.

The writer compared three of his cultures and one obtained from Dr. Valleau on the following media recommended by Sherbakoff (70): a hard oat agar (without glucose), a stem and tuber plug (beans and potatoes), and a potato agar with 5 per cent glucose. For convenience the cultures used will be designated as follows:

Strain number	Isolated from
I	Wheat seed from Missouri
II	Soil from St. Paul
III	Wheat roots from St. Paul
IV	Corn (?) from Kentucky (Dr. Valleau)

Marked differences between the strains appeared only on the potato agar. Strains II and III reacted similarly on this medium. Both caused the entire substratum to become deep reddish purple and both developed a light salmon-pink aerial mycelium. Strain I produced a similar growth on the surface of the agar but gave a purplish tint only near the surface of the substratum. Strain IV, on the other hand, failed to change the color of the substratum and produced practically white aerial mycelium. When the same strains were grown on sterilized wheat seed, I, II, and III produced macroconidia abundantly, but IV produced comparatively few in three tests made at different times. These results indicate that IV is probably distinct from I, II, and III, that II and III are very similar if not identical and differ only slightly from I.

It was considered that it might be possible to distinguish the strains on the basis of conidial length. An insufficient number of macroconidia was produced by Strain IV to make such measurements possible, hence Strains I and III were chosen for comparison. The measurements were necessarily made on conidia produced on one culture medium only, namely, sterilized wheat seed. Of course single spore cultures of the two strains were used and these were started at the same time on media prepared in the same way. The measurements of the mean length of the macroconidia in the different septation classes follow.

TABLE VIII

COMPARISON OF CONIDIAL LENGTHS OF TWO STRAINS OF *F. moniliforme*

Septation classes	Strain I—culture 48 days old			Strain III—culture 51 days old		
	Per cent of spores found	Length, microns	No. measured	Per cent of spores found	Length, microns	No. measured
1-septate	15	24.20	12	20	20.20	20
2-septate	8	30.30	9	4	25.60	4
3-septate	64	39.25 ± 0.39	100	71	38.02 ± 0.32	100
4-septate	8	42.90	15	3	43.10	3
5-septate	4	45.50	13	2	45.30	2
6-septate	1	49.20	2			

It is evident that only the 3-septate classes can be compared reliably, as an insufficient number of spores was found in the other septation classes. Moreover, since 3-septate spores are most typical of *F. moniliforme*, they would naturally be chosen as a basis for comparison. In this case the difference between the mean lengths of the 3-septate macroconidia of Strain I and Strain III is probably not significant, as it is less than three times the probable error of the difference.

It was found that Strains I, II, and III were equally virulent on wheat, while Strain IV was much weaker. Comparative data for Strains III and IV are given in Table IX and in Plate III, Figure 1. Series similar to those reported in Table VII were used.

TABLE IX

COMPARISON OF RELATIVE VIRULENCE OF TWO STRAINS OF *F. moniliforme* ON MARQUIS WHEAT

Strain	Length of test, days	Source of culture	Stunted seedlings	Seedlings with root-rot*	Seedlings with foot-rot	Mean height, cms.	Dry weight, gms.
III	38	Wheat roots	22/22	$22^{h}/22$	$12^{l}/22$	9.14	0.29
IV	38	Corn	18/64	$54^{l}/64$	$45^{tr}/64$	21.99	1.83
Control	38	....	11/91	0/91	0/91	27.81	5.03

* ^{h}=heavy, ^{l}=light, ^{tr}=trace.

Like results were obtained from two other similar tests. In one of these the inoculum was grown on sterilized soil to which 5 grams of cornmeal per 100 grams of soil had been added. The same relation was maintained, however, in respect to the relative pathogenicity of the two strains regardless of the kind of inoculum used, but the injury was of a somewhat lesser degree in the pots receiving the soil inoculum than in those receiving the seed inoculum, owing apparently to a less abundant growth of the fungus in the former. These results again indicate that Strain IV is different from Strains I, II, and III.[6]

The different strains of *Fusarium moniliforme* isolated by the writer were clearly pathogenic to wheat roots. The roots of seedling wheat plants were badly rotted when grown in inoculated agar, nutrient solu-

[6] These strains were sent to Dr. Sherbakoff for identification. He replied that he believed they were all of the *Fusarium moniliforme* species but considered that some of them would prove to be distinct organisms (presumably varieties or physiologic races).

tions, rag-doll germinators, and soil. The rotted roots were often badly stunted, especially in the inoculated soil (Plate IV). The tops of the diseased seedlings also were usually badly stunted. Both the stele and the cortex were attacked in the diseased roots examined. The former often showed indications of rotting before the latter. Both eventually became thoroly riddled with mycelium and then disintegrated. (Plate IX, Figs. 5 and 6.) The rules of proof were, of course, carried out. The foot of the plant, especially the part above ground, was apparently much less susceptible to attack than the roots. The coleoptiles of the seedlings were often attacked but the stem was usually not attacked until after the plants were greatly weakened by root-rot. Wheat seedlings from two to three weeks old were inoculated on the foot near the surface of the soil, but none developed foot-rot. Six seedlings were wounded before inoculation and four of them died, but it is doubtful if their death was due to the effects of the fungus. On wheat seedlings, therefore, *F. moniliforme* seems to be primarily a root-rotting fungus.

While most of the pathogenicity trials with *F. moniliforme* and with other fungi were made primarily on the seminal roots, a few adventitious roots frequently developed and became diseased before the conclusion of the tests, altho they seemed to be more resistant to most pathogenes than the seminals. Hence seedlings which survived long enough to produce them showed a tendency to recover. This was true even of plants infected with *Gibberella saubinetii*.

Experiments were made to determine whether *F. moniliforme* from wheat would attack other cereals. Table X and Plate V, Figures 1 to 3, show the results of growing wheat, oats, barley, rye, field corn, and sweet corn on soil inoculated with a culture of *F. moniliforme* (Strain I) growing on wheat seed. In these experiments two kinds of checks were used: those to which sterile medium was added and those to which none was added. Wheat, rye, oat, and field corn seedlings in the former type of check grew somewhat less vigorously than those in the latter type (Table X) but these differences were not marked (Plate V). The checks in all cases, however, were much superior to the seedlings in inoculated soil.

Each of the inoculated cereals was attacked by the fungus, which was readily recovered from surface-sterilized portions of the diseased roots, rhizome,[7] or foot of each, and abundant mycelium was found in the diseased tissues. The roots were severely rotted in all cases. Corn was severely attacked at the base of the plants as well, and the rhizomes of the oat plants were often thoroly rotted. The coleoptiles of the other seedlings were frequently discolored, but the stems apparently were invaded only after the plants had become weakened.

[7] Used in the same sense as Percival (loc. cit.-p. 7) used it.

TABLE X

EFFECT OF INOCULATING DIFFERENT CEREALS* WITH *Fusarium moniliforme* FROM WHEAT SEED

Cereal	Soil treatment	Length of test, days	Stunted seedlings†	Seedlings with root-rot	Seedlings with foot-rot	Mean height, cms.	Total dry weight, gms.
Wheat (Marquis)	Control. No medium added	32	0/23	0/23	0/23	28.94	0.77
" "	Control. Medium added	"	2/25	17/25	0/25	27.39	0.74
" " "	Inoculated with F.m.	"	15/20	20/20	18/20	12.84	0.23
" "	" " "	"	17/17	17/17	15/17	6.72	0.10
Barley (Improved Manchuria)	Control. No medium added	32	1/25	0/25	0/25	20.94	0.52
" " "	Control. Medium added	"	2/24	1/24	0/24	18.79	0.54
" " "	Inoculated with F.m.	"	16/16	16/16	9/16	7.23	0.19
" " "	" " "	"	21/21	21/21	11/21	6.68	0.19
Rye (Winter Swedish)	Control. No medium added	32	0/25	0/25	0/25	23.22	0.64
" " "	Control. Medium added	"	1/21	12/21	0/21	24.63	0.56
" " "	Inoculated with F.m.	"	16/19	19/19	17/19	9.49	0.21
" " "	" " "	"	12/16	16/16	8/16	11.69	0.19
Oats (Iowa 103-531)	Control. No medium added	32	2/25	0/25	0/25	20.54	0.32
" " "	Control. Medium added	"	5/22	3/22	0/22	18.20	0.27
" " "	Inoculated with F.m.	"	21/21	21/21	21/21	10.72	0.13
" " "	" " "	"	21/25	25/25	25/25	10.43	0.16
Field corn (Minn. No. 13)	Control. No medium added	26	0/10	0/10	0/10	39.2	2.30
" " " " "	Control. Medium added	"	0/9	0/9	0/9	37.2	1.77
" " " " "	Inoculated with F.m.	"	4/4	3/4	3/4	19.9	0.41
" " " " "	" " "	"	9/9	7/9	7/9	12.5	0.44
Sweet corn (Golden Bantam)	Control. No medium added	26	0/8	0/8	0/8	24.8	0.98
" " " "	Control. Medium added	"	0/8	4/8	5/8	24.4	1.23
" " " "	Inoculated with F.m.	"	4/4	1/4	4/4	9.7	0.12
" " " "	" " "	"	3/3	2/3	2/3	7.3	0.09

* Twenty-five seeds per pot of wheat, barley, rye and oats, and ten seeds per pot of corn, were sown.

† The denominator represents the number of seeds which produced plants, and the numerator the number stunted or affected with root- or foot-rot.

It is probable that *F. moniliforme* woud be much less destructive in the field than in these tests, as the amount of inoculum would rarely, if ever, be as abundant under field conditions. In old corn fields, however, *F. moniliforme* must be very prevalent on stubble, old stalks and roots, and even in the soil. It would be expected, therefore, that when wheat or some other cereal was sown on such land its roots would be attacked by this fungus. It is well known that *Gibberella saubinetii* occurs very abundantly in such fields and that wheat following corn is particularly heavily attacked by this fungus. It is possible that *F. moniliforme* may be destructive under similar conditions and that proper crop rotation will help to control it.

Other Fusarium spp.—Two other species of *Fusarium* studied also were decidedly pathogenic to wheat roots (Table VII, Series 29 and 45). Cultures of these were submitted to Dr. Sherbakoff, and he placed them in the section Elegans but has not yet been able to make specific determinations. These species attacked wheat roots in practically the same way as does *Gibberella saubinetti*, but were somewhat less virulent. They usually killed more of the seedlings than did *F. moniliforme*.

Several other species of *Fusarium* were slightly pathogenic on wheat roots in the greenhouse, e.g., *F. lini* (Table VII, Series 31). Many, however, such as *F. betae* (Table VII, Series 78) evidently are unimportant as pathogenes of wheat roots.

HELMINTHOSPORIUM SPP.

Helminthosporium sativum P.K. and B.—The pathogenicity of *H. sativum* on wheat roots has been definitely established by Stakman (75), Stevens (79, 81), Christensen (13), and Dosdall (19). All the strains studied by the writer were parasitic, but one isolated from a wheat seed from Oklahoma was particularly virulent and was used for comparison with other fungi. All the roots of wheat seedlings grown in soil inoculated with this strain were usually attacked. Diseased roots showed general or local browning and often were more or less speckled with minute darker spots over the surface of the rotted portions. Sometimes the rotting involved the whole root but it was often confined to the upper parts or to local areas. Some of the roots were frequently badly stunted by the fungus. The mycelium was found mainly in the cortex. Dark brown lesions frequently occurred on the rhizome, on the foot, and on seedling leaves. Diseased seedlings were usually stunted, altho some of them developed quite normally. In general, however, the stunting was less pronounced and the seedling survival greater than in soil inoculated with pathogenic species of *Fusarium*.

McKinney (55) reported that all the strains of *Helminthosporium* which he isolated from wheat were similar and corresponded morpho-

logically with *H. sativum*. Melchers (83) found at least two species of *Helminthosporium* on wheat but did not state how they differed. Drechsler (20) points out that several species other than *H. sativum* have been found on wheat, e.g., *H. geniculatum*, a small geniculate spored form reported by Palm from Java; *H. tritici* Hennings, a small-spored form differing from the former, collected by Zimmerman in the territory which was formerly German East Africa; a geniculate spored form reported by Stevens (79), in Illinois; and a few small-spored forms reported by Stakman (75), in Minnesota. The writer isolated at least four species (Plate XI) and probably five or more from wheat.

Helminthosporium N.—A *Helminthosporium* species differing from *H. sativum* sufficiently to be regarded as another species was isolated several times from wheat, especially from the seeds. It produced symptoms similar to those caused by *H. sativum* on the roots, foot, leaves, heads, and seeds of wheat, altho all strains tested were less virulent than *H. sativum*. A single spore culture of this organism produced conidia of rather uniform shape but differing considerably in length and number of septations. The conidia resemble those of *Podosporiella verticillata*, O'Gara, a fungus which O'Gara (57) described on germinating wheat seed. No synnemata, however, have been observed in the author's cultures even on wheat and rice, the media on which O'Gara always obtained normal fruiting.[8] The spores are much narrower and more cylindrical than the conidia of *H. sativum*. Data on conidial length, breadth, and number of septations of 100 spores of a single spore culture are compared with similar data for *H. sativum* in Table XI. (See also Plate XI, Figures 1 and 3.) The data on conidial width are also illustrated graphically in Figure 1, page 36. Cultures of the same age grown on sterilized mature wheat heads were used, and care was taken to avoid unconscious selection of the spores measured.

The most pronounced difference is in the width of conidia. This difference is more than forty-two times its probable error. There also is considerable difference in conidial length. It is noteworthy that the conidia of *Helminthosporium N* are shorter than those of *H. sativum*. When the two are examined in the same mount, however, this difference is not very apparent, as the conidia of *Helminthosporium N* look long, probably because they are so much narrower than those of *H. sativum*. No significant difference was found between the mean number of septations of the two, but *H. sativum* was more variable in this respect than *Helminthosporium N*.

[8] As the production of synnemata seems to take place only under certain conditions, it has seemed advisable to study this fungus further before deciding on its identity. Dr. Drechsler, who examined a culture of the fungus, considered it distinct from *H. sativum* and also noted the similarity of the conidia with those of *Podosporiella verticillata*. He observed light areas at the ends of the spores which were larger than those in *H. sativum* and fuliginous rather than dark olivaceous in color.

TABLE XI

VARIATIONS AND CONSTANTS FOR CONIDIAL MEASUREMENTS OF *Helminthosporium N* and *Helminthosporium sativum*

a. Conidial lengths in microns

Fungus	Spore classes									Total No.	Constants			
	15	25	35	45	55	65	75	85	95		Mode	Mean	Standard deviation	Coefficient of variability
Helminthosporium N	3	2	6	6	19	24	19	18	3	100	65	64.20±1.21	17.93±.86	27.92±1.33
Helminthosporium sativum	1	5	3	4	5	15	29	32	6	100	85	71.40±1.21	17.97±.86	25.16±1.20

b. Conidial widths in microns

Fungus	Spore classes										Total No.	Constants			
	9	11	13	15	17	19	21	23	25	27		Mode	Mean	Standard deviation	Coefficient of variability
Helminthosporium N	3	8	36	45	5	3	..	..	..	..	100	15	14.00±.13	1.87±.09	13.32±.64
Helminthosporium sativum	..	1	..	..	3	6	23	13	45	9	100	25	23.26±.19	2.75±.13	11.82±.56

c. Conidial septations

Fungus	Spore classes												Total No.	Constants			
	1	2	3	4	5	6	7	8	9	10	11	12		Mode	Mean	Standard deviation	Coefficient of variability
Helminthosporium N	..	2	5	5	4	9	24	22	21	6	2	..	100	7	7.28±.13	1.96±.09	26.92±1.28
Helminthosporium sativum	2	4	2	2	8	11	24	18	15	9	4	1	100	7	7.23±.15	2.25±.11	31.12±1.48

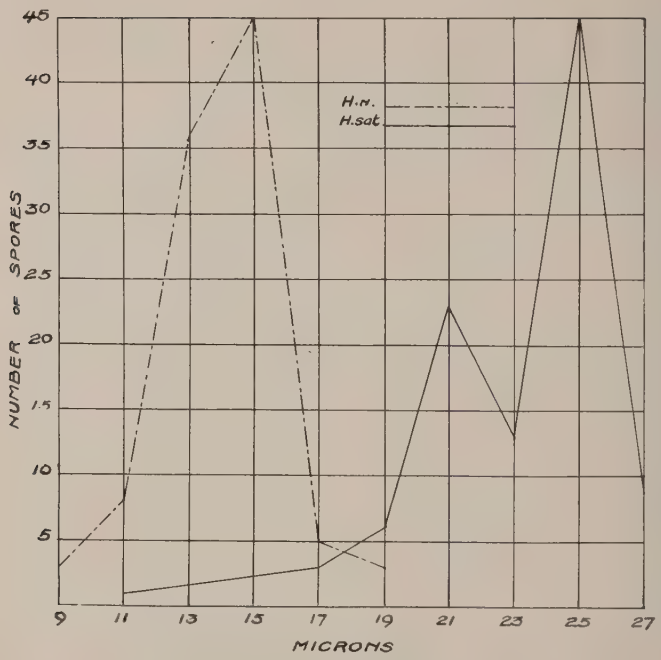

Fig. 1. Relative Conidial Width of Helminthosporium N and H. sativum

Helminthosporium M.—Several similar small-spored strains are included here. These were isolated from several sources, as indicated below. For convenience we shall refer to them by number as follows:

Strain	Isolated from
I	Wheat seed from St. Paul, Minn.
II	" " " Burdette, Kan.
III	" " " Indian Head, Sask.
IV	Millet leaf from Waukegan, Ill.

Two other similar small-spored strains were also isolated, one from wheat roots and one from barley roots, but these were not studied further. The relative pathogenicity, spore dimensions, and reaction on different culture media of Strains I, II, III, and IV, however, were studied in some detail.

The exact identity of these forms is difficult to determine. They apparently may be placed with justification in either of two genera, namely, *Helminthosporium* or *Brachysporium*. They were previously tentatively assigned to the latter genus (31) in order to distinguish them from certain large-spored species of *Helminthosporium* from

wheat (Plate XI). If Lindau's (44) suggestion were followed, namely, that *Brachysporium* be reserved for species whose spores are not more than twice as long as they are broad, then three of the above strains would belong in the genus *Helminthosporium* and one in *Brachysporium*. This suggestion, however, has not been followed in the case of many species of *Brachysporium*, e.g., *Brachysporium graminis* Boy. and Jacz. (67) and *Brachysporium trifolii* Kauffman (9). The latter species and also *Helminthosporium inaequalis* Shear (74) have spores which correspond rather closely in shape and size to the conidia of the forms isolated by the writer.

These small-spored cultures were submitted to Dr. Charles Drechsler for identification. He was unable to place them specifically but preferred to refer them to the genus *Helminthosporium* rather than to *Brachysporium*.[9] Stevens (81) also placed several similar small-spored forms isolated from corn, in the genus *Helminthosporium*. In order to avoid confusion, the small-spored forms from wheat, barley, and millet are therefore also referred to this genus in this paper. Two of these strains, namely, III and IV, produced the erect sclerotoid coremia-like bodies in old cultures similar to those described by Shear, for his *H. inaequalis*. It is possible that they are closely related to that fungus, but Dr. Drechsler was of the opinion that they were not identical with it.

In order to compare the cultural characters of the four strains, they were grown on three different media: Oat agar, Czapek's agar, and potato dextrose agar. Cultures from single spores were used in all cases. Duplicate plates of each medium were inoculated with each strain. The plates were inoculated in the center with a small bit of mycelium, and notes were taken on the cultures at the end of the third and sixth days. Invariably, duplicate colonies of the same strain on the same medium were alike. These results are presented in Table XII and Plate VI. Strain I produced an orange pigment on Czapek's agar and on potato dextrose agar, while none of the others did. Strain II could also be readily distinguished from the others on these agars by its uniformly dark, abundantly sporulating colonies. The strains resembled each other most on oat agar, but even on this medium they could be distinguished. Strains III and IV could be distinguished on Czapek's agar, but less readily on potato dextrose agar. In general, Strains III and IV were most nearly alike. As will be pointed out later, however, the pathogenicity of these two strains differed.

[9] Dr. Drechsler considered that the only way to deal with these forms was to collect large numbers of strains and then to separate them into their component species, as it is impossible without further study to apply either a new or an old binomial to them.

TABLE XII

COMPARISON OF GROWTH AND CULTURAL CHARACTERS OF FOUR STRAINS OF *Helminthosporium M*

No.	Age of culture, days	Average diameter of colonies, cms.			Cultural characters of colonies		
		Oat agar	Czapek's agar	Potato dextrose agar	Oat agar	Czapek's agar	Potato dextrose agar
I	3	3.66	4.73	4.13	Colonies fuscous in center, mostly subepidermal dusky olive green. Trace of white aerial mycelium. Spores few, in center only.	White aerial mycelium in center. Outer zone aniline yellow mostly within substratum. White border. Spores few.	Dense growth of gray to white aerial mycelium in center and covering most of the colony. Orange border. Spores few.
	6	6.00	7.96	6.53			
II	3	4.36	5.23	4.96	Olive green to black colony with light border. Sporulating heavily over whole surface. Aerial growth dark, with radiations.	Olive green to black colony. Aerial mycelium uniform over surface, dark, powdery with spores.	Olive green to black except for a light gray border. Aerial growth dark with radiations. Sporulating heavily over entire surface.
	6	7.23	8.30	7.50			
III	3	4.26	4.86	4.30	Deep slate green. Aerial growth mostly dark, not fluffy. Spores plentiful over whole surface.	Center deep slate green from below, with small circle of white aerial mycelium above. Outer zone light gray turning slate green with age, fluffy. Spores moderately abundant all over colony.	Colonies gray turning deep slate green with age. Aerial mycelium fairly uniform but becoming densest in center with age. Spores moderately abundant. Slight zonation.
	6	7.30	7.86	6.80			
IV	3	4.36	5.06	4.36	Deep slate green. Aerial mycelium fluffy, abundant, sporulating medium to abundant over whole surface.	Fluffy aerial mycelium over whole surface. Colony fuscous in center bordered with a light gray zone. Spores abundant in center of colony.	Colonies gray turning deep slate green with age. Aerial growth gray becoming more prominent near edge with age. Spores moderately abundant. Slight zonation.
	6	7.33	8.50	6.93			

TABLE XIII

VARIATIONS AND CONSTANTS FOR CONIDIAL MEASUREMENTS OF FOUR STRAINS OF *Helminthosporium M.*

a. Conidial lengths in microns

Strain	Spore classes											Total No.	Constants			
	9	12	15	18	21	24	27	30	33	36	39		Mode	Mean	Standard deviation	Coefficient of variability
I	..	1	..	3	11	24	29	20	10	1	1	100	27	26.61±.29	4.28±.20	16.08±.77
II	..	3	1	7	29	49	11	..	..	..	..	100	24	22.59±.21	3.04±.14	13.47±.64
III	..	..	..	5	11	21	31	21	6	5	..	100	27	26.70±.29	4.23±.20	15.85±.76
IV	1	2	1	3	17	25	28	20	2	..	1	100	27	25.20±.31	4.55±.22	18.06±.86

b. Conidial widths in microns

Strain	Spore classes										Total No.	Constants			
	7	8	9	10	11	12	13	14	15	16		Mode	Mean	Standard deviation	Coefficient of variability
I	..	2	5	14	22	24	21	8	3	1	100	12	11.77±.11	1.57±.07	13.32±.64
II	1	1	3	7	20	30	27	10	..	1	100	12	11.99±.10	1.41±.07	11.76±.56
III	1	2	8	24	29	21	11	4	..	..	100	11	11.05±.09	1.38±.07	12.49±.59
IV	1	8	10	30	29	18	2	2	..	..	100	10	10.50±.09	1.33±.06	12.66±.60

c. Conidial septations

Strain	Spore classes						Total No.	Constants			
	1	2	3	4	5	6		Mode	Mean	Standard deviation	Coefficient of variability
I	1	2	32	62	2	1	100	4	3.65±.04	.65±.03	17.92±.85
II	3	6	91	..	..	..	100	3	2.88±.03	.41±.02	14.13±.67
III	..	1	23	75	1	..	100	4	3.76±.03	.47±.02	12.54±.60
IV	2	2	22	73	1	..	100	4	3.69±.04	.63±.03	17.01±.81

In order to obtain material for comparing the spore dimensions of the four strains, single spore cultures were grown on sterilized mature wheat heads in test tubes under as uniform conditions as possible. The spores were measured in a water mount, with a screw micrometer. Unconscious selection of spores for measurement was avoided by measuring only those which lay parallel to one of the cross hairs of the micrometer, and by measuring all such spores that were encountered in passing from one side of the mount to the other. The length, breadth, and number of septations of each spore were recorded. One of the longest-spored strains was selected for measurement first, in order to determine how many spores it would be necessary to measure in order to avoid errors which might result from measuring an insufficient number. It was found that the differences between the mean lengths of 50, 100, 150, and 200 spores were insignificant. It was decided, therefore, to measure 100 spores of each strain. It was also found that there were no significant differences between the means of different lots of 50 spores and different lots of 100 spores from the same culture. Table XIII summarizes the data from these measurements.

Two of the strains from wheat seed, namely, I and III, have practically the same means for conidial length, but the conidia of Strain II are much shorter, the difference between II and I being 11.23 times the probable error of the difference. Spores of Strain IV are also somewhat shorter than those of Strain I, the difference in this case being 3.32 times its probable error. In mean conidial width, I and II do not differ significantly, while III and IV differ from each other and also from both I and II. The extremes, as represented by I and IV, present a difference which exceeds its probable error 11.12 times. The mean number of septations in I, III, and IV agree closely; their spores are normally 4-septate. Those of II, however, are typically 3-septate. The relative conidial lengths of the four strains are illustrated graphically in Figure 2, page 41. Strain II stands out from the others in having shorter spores with fewer septations.

Strains I and IV were rather virulent on wheat, while Strains II and III were only weakly parasitic (Plate VIII, Fig. 1; also Plate VII, Fig. 1). The effect of the different strains on two varieties of wheat Marquis and Kanred, grown on sterilized soil inoculated with cultures of the organisms, is shown in Table XIV. All strains attacked the roots, invading the cortex as far as the endodermis. (Plate IX, Figs. 3 and 4.) The mycelium was most abundant in the intercellular spaces, altho frequently it was intracellular, some host cells being packed full of the large, dark brown hyphae. Infected portions were often nearly black, owing to the abundance of the dark mycelium in the cortex. Others showed symptoms much like those produced by *H. sativum*. In

the case of the more virulent strains (I and IV), roots were frequently attacked throughout their length and were badly stunted, whereas the attack of the other strains was usually more localized. Strains I and IV also produced more pronounced stunting of the tops and frequently dark brown spots on the first seedling leaves, as well as foot-rot similar to that produced by *H. sativum*. The order of virulence of the four strains on the heads and grains was the same as on the seedlings. All strains were re-isolated many times from diseased roots.

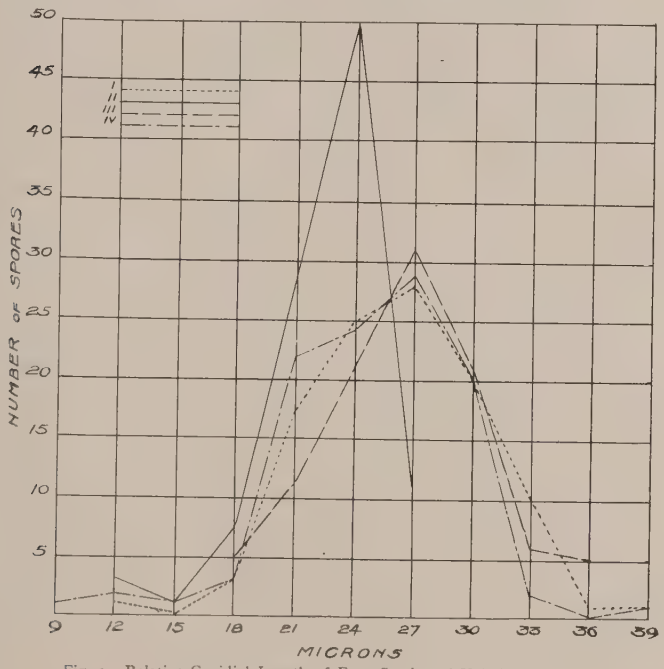

Fig. 2. Relative Conidial Length of Four Strains of Helminthosporium M

TABLE XIV

COMPARATIVE EFFECT OF FOUR STRAINS OF *Helminthosporium M* ON TWO VARIETIES OF WHEAT

Strain	Variety of wheat	Duration of test, days	Stunted seedlings	Seedlings with foot-rot*	Seedlings with root rot*	Mean height, cms.	Total dry weight of tops, gms.	Observations
I	Marquis	38	20/64	$64^{h}/64$	$64^{l}/64$	15.88	1.32	12% dead
I	Kanred	"	60/60	$60^{h}/60$	$28^{l}/60$	8.84	0.92	28% dead
IV	Marquis	"	44/44	$44^{h}/44$	$36^{m}/44$	11.00	0.68	12% dead
IV	Kanred	"	44/68	$68^{h}/68$	$28^{m}/68$	10.50	0.84	16% dead
II	Marquis	"	4/64	$64^{l}/64$	$8^{l}/64$	27.10	3.36	4% dead
II	Kanred	"	32/88	$88^{l}/88$	0/88	21.22	4.64	
III	Marquis	"	20/84	$84^{l}/84$	$12^{tr}/84$	20.74	2.56	
III	Kanred	"	32/76	$72^{l}/76$	$8^{tr}/76$	19.45	2.36	
Control	Marquis	"	11/91	$0^{tr}/91$	0/91	27.81	5.03	

*^{h}=heavy, ^{m}=moderately heavy, ^{l}=light, ^{tr}=trace.

If there should be in these strains but one collective species or a Linnean, as Stevens (81) and Christensen (13) found with *H. sativum*, it would at least comprise three if not four elementary species. The spores of the four strains have the same general morphology. They are dark olivaceous, usually curved (Plate XI, Fig. 2), each with a small hilum at the lower end, borne in a similar manner (Plate XII, Fig. 5), and germinating usually from both ends (Plate XII, Figs. 1 and 2). Differences in size, however, are found when careful measurements are made and possibly other morphological differences will be found with closer study. It may be that they should be designated as distinct species, but no binomial can be applied until the whole group of small-spored *Helminthosporia* has been studied more carefully.

Inoculation experiments also were made with *H. gramineum* (Rab.) Erik. and *H. teres* Sacc. While the results were somewhat inconclusive, neither appeared to be a virulent parasite of wheat roots.

Helminthosporium sp.—Another very interesting species of *Helminthosporium* was isolated from a diseased wheat root growing in the greenhouse on a continuous-wheat soil obtained from one of the agronomy field plots. This fungus caused dark brown lesions on wheat roots in greenhouse tests and the mycelium penetrated the cortex, but it was only weakly parasitic, if at all. It was readily recovered from the roots. The spores and conidiophores are shown in Plate XI, Figure 4. It is readily seen that the fungus differs from any of the other species of *Helminthosporium* isolated from wheat.[10] It constitutes a well differentiated species which apparently has not been previously described. A new species is therefore proposed with characters as follows:

Helminthosporium pedicellatum n. sp.—Producing, on potato dextrose agar, fine hyaline aerial mycelium with a coarser fuliginous mycelium in the substratum; conidiophores clavate, rarely branched, relatively straight to the point of attachment of the first spore, above which successive spores are produced at the apices of geniculations; measuring 2.5 to 9.5 μ in diameter, producing the first spore usually 100 to 200 μ from the base and developing from 2 to 9 septations below the first spore; total length very variable; brownish olive and thick-walled above but usually lighter and thinner walled towards the base; points of attachment of spores marked by scars. Conidia broadly fusiform, typically straight, widest near the middle, and decidedly attenuated toward both ends, being rounded off at the apex at approximately one-sixth to one-third the maximum width of the spore tho occasionally much broader at the apex, narrowed to a pedicel-like elongation at the base, slightly constricted at the hilum; brownish olive episporium with

[10] A culture was submitted to Dr. Charles Drechsler who considered it a well characterized species, different from any that he had previously seen.

small pale area adjacent to the hilum, hyaline gelatinous endosporium; germinating from both ends but usually from the base, 1-9 septate but usually 7 septate; measuring 23.42±0.17 by 65.30±0.96 (14.71 to 29.44 by 31.00 to 91.44); measurements based on 100 spores grown on a sterilized mature wheat head.

Isolated from wheat roots at St. Paul, Minn., Sept. 28, 1922.

MISCELLANEOUS FUNGI

Other genera contain species which can attack wheat roots but not to such an extent as to interfere markedly with the development of the plants. While they are interesting, it is not considered that they would be very destructive under field conditions. Only a few of them therefore will be mentioned.

Alternaria.—Many strains of this genus were tested but the results were conflicting. In the first series of tests made in test tubes, 47 strains were used, and a few apparently were parasitic. Inoculations were made with the apparently parasitic forms, but the results were variable. All the strains tested in the greenhouse were only weakly parasitic. Diseased portions of the roots were usually localized and brown to black in color, and in these regions the mycelium was found in the cortex only. The fungus was re-isolated frequently.

Stemphylium parasiticum (Thüm.) Elliot.—This fungus, altho able to produce black-point on the seeds, failed to attack the roots to any appreciable extent. Occasionally tiny black lesions were formed on the roots, but usually inoculated roots remained perfectly white and uninjured. The morphology of the fungus corresponds rather closely with that of the species appearing in literature as *Macrosporium parasiticum* Thüm (23). Following Elliot (22), however, this becomes *Stemphylium parasiticum* (Thüm.) Elliot. According to Miyabe (51), *M. parasiticum* and *M. sarcinula* Berk. are identical and *Pleospora herbarum* (Pers.) Rab. is the perfect stage. However, the latter stage has not yet been observed in the writer's cultures.[11] *M. commune* Rab. has also been considered identical with *M. sarcinula* (44) and *M. parasiticum* (80), but Lindau (44) preferred to keep the three species separated until further work confirmed their identity.

Altho the spores (Plate XII, Figs. 6 and 7) of the culture on wheat are similar in shape to those of *M. sarcinaeforme* Cav. on clover (42), they differ in size and also in having a rough exospore. *Stemphylium tritici* Pat. (58) has been reported by Johnson (37) as the cause of

[11] For this reason it has been deemed advisable not to use the name *Pleospora herbarum* (Pers.) Rab. until the perfect stage of the writer's material has been found and proved to belong to this species. Altho *M. sarcinula* Berk. was described before *M. parasiticum* Thüm., the latter specific name is used because authentic exsiccati material of Berkeley's fungus was not available and the writer's fungus corresponded closely with von Thümen's specimen of *M. parasiticum* (667 de Thümen Mycotheca Universalis).

floret sterility of wheat, but this undoubtedly is a different species, as the spores are catenulate.

Phoma sp.—An interesting species of *Phoma* was obtained several times from a continuous-wheat soil, at University Farm, St. Paul. After a week or more in culture, this fungus developed abundant pycnidia and numerous dark brown chlamydospores in the mycelium. Thinking that the latter might belong to another fungus, cultures were several times secured from single pycnospores, but invariably these cultures developed typical chlamydospores. This fungus in inoculated soil readily penetrated the root cortex as far as the endodermis, and frequently the cortex was literally riddled with its mycelium (Plate IX, Figs. 1 and 2). The presence of the fungus was very evident from the fact that the mycelium produced many large chlamydospores. These were observed in root hairs and in epidermal and cortical cells, but never in cells of the central cylinder. The attacked parts were sometimes several centimeters in length, and almost black, but rarely extended to the ends of the roots, being mostly within an inch or two of the seed. Many re-isolations were made from affected roots.

Rhizoctonia.—Stakman (76) inoculated durum wheat seedlings with this fungus. It caused a general blighting of the seedlings but attacked the roots only slightly. The writer did not isolate this fungus in the field. However, in two instances, tiny black sclerotium-like bodies developed on the roots of check plants growing in nutrient agar. *Rhizoctonia* was isolated from these. The fungus could have come from no other source than from the seed, in these instances. Several seedlings were inoculated in test tubes with the fungus, and the sclerotium-like bodies developed on the roots of the inoculated plants, whereas roots of the controls remains normal. The seedlings were also attacked at the foot and some were severely blighted. Greenhouse tests were inconclusive. No sclerotium-like bodies developed on the roots, and the plants grew quite normally.

Repeated unsuccessful attempts were made to isolate the fungus from what appeared to be *Rhizoctonia* mycelium, in tiny spots about 1 mm. long, occurring very abundantly on wheat roots in unsterilized soil, both in the field (Plate II, Fig. 2) and in the greenhouse. The mycelium was usually confined to a few epidermal cells, and often formed a small sclerotium-like body on the exterior. Sometimes the whole root was encircled by the mycelium, which is large, dark brown, and somewhat resembles that of *Rhizoctonia*. No rotting was evident and the fungus appeared to cause very slight injury, if any.

Colletotrichum graminicolum (Ces.) Wilson. — In this species Wilson (7) includes several forms that were previously described

as distinct species, e.g., *Colletotrichum cereale* Manns. This fungus was only weakly parasitic on wheat roots in the greenhouse and was not studied further.

Aspergillus niger Van Tiegh.—A strain of this fungus, from wheat seed from South Dakota, was pathogenic in preliminary tests and moderately so in the greenhouse. The tips of the roots were rotted most noticeably and the fungus was recovered from the diseased parts. Other trains were non-parasitic.

Acrostalagmus sp.—Several similar cultures of a fungus answering the general description of *Acrostalagmus* (43) were isolated from wheat roots and from the soil. The spores were borne in slimy heads, altho sometimes they were in ear-like masses on verticillate conidiophores. In the young cultures, however, the spores were typically in slimy heads. While this fungus was not particularly destructive, it did cause a characteristic deformity of the roots of some of the wheat seedlings grown in inoculated soil (Plate VIII, Fig. 2). Such roots were stunted, swollen, gnarled, and rotted particularly at the tips. The tops of these diseased plants were invariably badly stunted. However, most of the plants in pots of inoculated soil developed normal or nearly normal root systems. The fungus was readily recovered in pure culture from the diseased roots, but there is little reason to think that it would be destructive under field conditions.

Trichoderma spp.—Several strains of *Trichoderma* which were not identified specifically were slightly parasitic in the greenhouse, but did not appear sufficiently destructive to merit special study. One of these resembled *Trichoderma lignorum* (Tode) Harz., as described by Cook and Taubenhaus (15), altho it may not be identical with it. The others were more similar to *T. koningi* Oudemans which Stakman (76) found to be parasitic on cereal roots.

Other fungi.—Several cultures of a Cephalosporium, resembling *Cephalosporium acremonium* Cda., as well as a few forms which failed to produce conidia, were either non-parasitic or very weak pathogenes in the greenhouse. In addition, many non-pathogenic species were discarded in the preliminary tests. (See Table VI.)

COMPARISON OF THE VIRULENCE OF DIFFERENT FUNGI ON WHEAT

Several fungi were selected which had shown varying degrees of virulence in previous trials. Each was grown on an equal amount of sterilized wheat seed medium, and all cultures were started at the same time. After the medium of the different cultures was thoroly permeated with mycelium, each culture was used to inoculate a pot of sterilized soil in the greenhouse. Twenty seeds of winter wheat (Minhardi,

Minn. 1505) were sown in each pot. The results of this experiment are given in Table XV. (See also Plate V, Fig. 4.)

TABLE XV

COMPARATIVE EFFECT OF SOIL INOCULATION WITH DIFFERENT FUNGI ON WINTER WHEAT (MINHARDI, MINN. 1505) IN THE GREENHOUSE

Inoculum	Duration of test, days	Stunted seedlings*	Seedlings with root-rot†	Seedlings with foot-rot	Mean height, cms.	Total dry weight of tops, gms.
None (control)	34	2/20	0/20	0/20	26.54	0.51
" "	"	0/17	0/17	0/17	30.03	0.48
" "	"	0/15	1/15	1/15	28.55	0.31
" "	"	1/18	1/18	0/18	27.10	0.43
Stemphylium parasiticum	"	0/19	0/19	7/19	30.00	0.54
Helminthosporium pedicellatum n. sp.	"	2/18	$18^{l}/18$	2/18	24.19	0.39
Helminthosporium sativum	"	3/12	$12^{h}/12$	2/12	23.60	0.21
Helminthosporium M. Strain I	"	1/13	$13^{h}/13$	0/13	19.73	0.19
Fusarium moniliforme (from seed)	"	10/12	$12^{h}/12$	0/12	12.86	0.13
Fusarium moniliforme (from soil)	"	12/14	$14^{h}/14$	3/14	11.88	0.13
Gibberella saubinetii (soil)	"	3/6	$6^{h}/6$	3/6	13.26	0.07
Fusarium sp. (soil) (Elegans section)	"	No plants emerged from the soil				
Gibberella saubinetii (seed)	"	" "	" "	" "		
Fusarium sp. (foot) (Elegans section)	"	" "	" "	" "		

* The denominator indicates the number of seeds (out of 20) which produced plants. The numerator represents the number of surviving seedlings which were stunted or attacked by root- or foot-rot.

† ^{l}=light. ^{h}=heavy.

In the field, duplicate rod rows were also inoculated in a similar way with cultures of several of the fungi, and sown with both winter and spring wheat. The winter wheat (Minhardi, Minn. 1505) was sown on September 30, 1922, and the spring wheat (Marquis) on May 15, 1923. Notes were taken on the seedlings in each case about six weeks after planting. The percentage seedling survival was taken as an index of the relative virulence of the fungi. The results of these tests are shown in Table XVI.

In the greenhouse the *Fusarium spp.* were more virulent than the *Helminthosporium spp. Stemphylium parasiticum* and *Helminthosporium pedicellatum*, on the other hand, caused little if any injury to the seedlings. After considering the field results also, it is quite evident that *Gibberella saubinetii* is the most virulent parasite tested. This was true not only in the fall when soil temperatures were low but also in the spring when they were relatively high. *Helminthosporium sativum* was also decidedly destructive in the fall, but unfortunately was not included in the tests made in the spring. *Helminthosporium M.* Strain 1, while equally as destructive as *Helminthosporium sativum* in the greenhouse, was less so in the field. *Fusarium moniliforme* apparently destroyed nearly as high a percentage of seedlings under high soil tempera-

ture conditions (spring) as *Gibberella saubinetii,* but was much less virulent in the fall and somewhat less so in the greenhouse. The two *Fusarium spp.* of the Elegans section were much less destructive in the field than in the greenhouse. The number of seedlings killed, of course, represents only a part of the injury. Naturally most of those that grew were weakened in varying degrees. In further tests it obviously would be desirable to have at least one series on sterilized soil.

TABLE XVI

SEEDLING SURVIVAL OF WINTER AND SPRING WHEAT SOWN IN ROD ROWS AFTER INOCULATING THE SOIL WITH DIFFERENT FUNGI

Inoculum*	Percentage of seedlings which survived					
	Winter wheat Minhardi, Minn. 1505)			Spring wheat (Marquis)		
	First series	Second series	Average	First series	Second series	Average
None (Control)	88.5	84.5	86.5	58.0	65.0	61.5
Helminthosporium sativum	35.0	32.0	33.5	...	...	...
Helminthosporium M Strain I	62.5	57.0	59.7	64.0	56.0	60.0
Fusarium moniliforme Strain I	69.5	73.5	71.5	...	...	...
Fusarium moniliforme Strain II	68.5	72.5	70.5	38.0	41.5	39.7
Fusarium sp. Elegans section (soil)	59.5	66.5	63.0	52.5	63.0	57.7
Fusarium sp. Elegans section (foot)	75.5	68.0	71.7	55.5	52.5	54.0
Gibberella saubinetii (roots)	21.5	20.5	21.0	...	...	...
Gibberella saubinetii (seed)	10.5	16.0	13.2	40.5	35.5	38.0

* A fairly high percentage of seedlings was killed in rows to which sterile uninoculated medium was added. This was apparently due to the fact that the soil used was not sterilized and hence the medium introduced formed a suitable substratum for the multiplication of pathogenes present in the soil. On sterilized soil in the greenhouse, on the other hand, there was usually only slight root injury to the control plants and little difference in this respect between controls sown in soil to which the sterile medium was added and those sown in soil to which none was added (see Table X and Plate V).

ANATOMY OF WHEAT ROOTS IN RELATION TO ROOT-ROT

In describing the anatomy of the seminal roots of wheat, Percival (60) distinguishes three principal regions, which are shown in cross-section: (a) a piliferous layer developing root hairs from many of its cells, (b) a broad cortical region, and (c) a central vascular cylinder or stele. The cortical region consists of several layers of thin-walled, parenchymatous cells with small intercellular spaces, and an inner layer or endodermis made up of closely packed cells whose outer walls are thin but whose inner walls become thickened and strongly cuticularized, particularly when old, and whose radical walls also become thickened especially toward the interior of the roots. The third region, the stele, has an outer layer, the pericycle, which consists of a single row of somewhat elongated, thick-walled, lignified cells, uniform in size except for smaller cells opposite the protoxylem elements. Within are seven or eight xylem strands, and alternating with them are the

phloem bundles. A large pitted vessel is found in the center of the stele; sometimes there are two or more. The conjunctive parenchyma of the stele also has rather thick walls.

One of the striking features noticed in the examination of sections of diseased roots is the localization of the mycelium of different fungi to particular regions of the root. The piliferous and cortical regions are most commonly attacked, hence we get roots with "loose cortex." In Plate IX, Figures 1 and 2, two sections of a seminal root attacked by *Phoma sp.* are shown. It will be noted that the stele is intact and free from mycelium, while the cortex is riddled with it. A similar condition is illustrated in Figures 3 and 4 of the same plate, which represent sections of roots attacked by *Helminthosporium M.* In both instances the mycelium has penetrated to, but not beyond, the endodermis. In Figures 3 and 4, one or two of the endodermal cells have been entered by the mycelium of *Helminthosporium M*, but no hyphae have penetrated the thick, lignified, inner endodermal walls. On the other hand, in Plate IX, Figure 5, a cross-section of a root is shown in which the central cylinder is primarily attacked. This root was infected with *Fusarium moniliforme*. Figure 6 illustrates a later stage, after the stele has disintegrated. By this time the cortex is also thoroly permeated with mycelium.

From the standpoint of injury to the roots, the latter type of attack is undoubtedly the more serious. It is true that the former may involve the whole root, or the actively absorbing part, and thus reduce its activities materially, but very often only a portion of the root is involved. Such a root may be completely girdled and appear to be cut off, and yet the central cylinder may be intact and the vascular elements may be functional. Lateral rootlets may even be sent out from the stele, through such diseased cortices, showing that it is alive and functioning.

Very evidently the closely-packed cells of the endodermis and their thickened inner walls form a natural barrier against the invasion of many fungi into the central cylinder. The structure and arrangement of the cells of the pericycle as well as those of the conjunctive parenchyma present further barriers to the progress of the mycelium of certain fungi. Percival (60) points out that in roots with decayed cortex, the endodermis, pericycle, and conjunctive parenchyma of the stele become very strongly sclerotic, while cells of the bast and xylem vessels preserve their normal character. Hence these roots would be all the more effectively protected against such decorticating fungi as *Phoma sp.*, *Helminthosporium spp.*, *Alternaria spp.*, etc. This is probably one reason why these fungi cause less pronounced stunting of wheat seedlings than certain species of *Fusarium*, which are able to attack the central cylinder of the roots.

PHYSIOLOGICAL STUDIES OF ROOT-ROTTING ORGANISMS

EFFECT OF TEMPERATURE ON GROWTH

It is particularly important to know the reaction of root-rotting fungi to temperature. Soil temperatures may determine whether or not certain fungi will be able to develop in the soil and attack the roots. Dickson (17), for instance, using *Gibberella saubinetii*, obtained severe blighting of wheat seedlings at soil temperatures of from 16-28° C., whereas no blighting occurred at soil temperatures below 12° C., very little at 32° C., and none at all at 36° C. He further states (18) that since the attack of the organism is chiefly on the young seedling, the range of temperature during the "nursing period" is undoubtedly the important factor in determining the severity of this stage of the disease.

As a preliminary step to soil temperature studies, it was considered advisable to determine first the effect of temperature on the growth of certain root-rotting organisms on culture media. Three fungi were chosen for study, namely, *Fusarium moniliforme* (Strain II); *Fusarium sp.*, Elegans section (soil); and *Helminthosporium M* (Strain I). Petri dishes (10 cm. in diameter) were each poured with 25 cc. of potato dextrose agar, and were inoculated at the center with bits of mycelium from single spore cultures of the different fungi. Duplicate plates of each fungus were then placed at several temperatures between 0 and 39° C. The relative growths at the different temperatures were measured by taking the mean diameters of the colonies on two different dates. The results of these experiments are presented in Tables XVII and XVIII, and also in Plate VII, Figure 2.

TABLE XVII

EFFECT OF TEMPERATURE ON GROWTH OF TWO SPECIES OF FUSARIUM ON POTATO DEXTROSE AGAR

Temperature range, degrees C.	Mean temperature, degrees C.	Diameter of colonies, mm. *Fusarium moniliforme* Strain II		*Fusarium sp.* Elegans section (Soil)	
		2 days	5 days	2 days	5 days
0.0 - 5.0	4.1	0	0	0.0	0.0
6.5–12.0	8.7	4	5	3.0	4.0
12.5–14.0	12.9	10	21	14.0	24.0
18.0–20.0	19.0	26	42	25.0	42.5
21.5–23.0	22.3	34	59	34.0	58.5
26.5–27.0	26.6	43	74	38.5	66.5
29.0–30.0	29.6	49	78	37.0	64.0
32.0 33.0	32.2	37	58	7.5	28.0
35.0–35.5	35.3	11	14	3.0	3.0
35.5–37.0	36.5	2	4	0.0	0.0
37.0 39.0	38.0	0	0	0.0	0.0

TABLE XVIII

EFFECT OF TEMPERATURE ON GROWTH OF *Helminthosporium M* (STRAIN I) ON POTATO DEXTROSE AGAR

Temperature range, degrees C.	Mean temperature, degrees C.	Diameter of colonies, mm.	
		4 days	6 days
0.0– 5.0	3.5	0.0	0.0
6.5– 8.0	7.6	3.0	6.0
13.0–15.0	13.9	15.0	22.0
18.0–22.0	19.6	26.0	41.5
21.5–24.5	22.8	31.5	50.2
26.0–28.0	27.0	37.5	57.7
29.0–31.5	29.8	39.0	63.0
31.5–33.5	32.0	41.5	53.0
35.0–35.5	35.3	7.0	11.5
36.0–37.0	36.9	7.0	7.5
37.0–39.0	38.0	0.0	0.0

The cardinal temperatures for growth of these three species of root-rotting fungi on potato dextrose agar are therefore approximately as follows:

	Minimum, degrees C.	Optimum, degrees C.	Maximum, degrees C.
Fusarium moniliforme (Strain II).....	6.5–12	29.0–30.0	35.5–37.0
Fusarium sp. Elegans section (Soil)...	6.5–12	26.5–27.0	35.0–35.5
Helminthosporium M. (Strain I).....	6.5– 8	29.0–31.5	36.0–37.0

All three species grow best at rather high temperatures. A distinct difference in growth reaction to temperature is shown by the two species of *Fusarium*. *F. moniliforme* will grow at considerably higher temperatures than *Fusarium sp.* (Compare especially their relative growth at 32.2°—Plate VII, Fig. 2.) Strain I of *F. moniliforme* was compared with Strain II at a series of temperatures corresponding to the above, but no differences could be detected between the behavior of the two strains at the different temperatures.

SOIL TEMPERATURE EXPERIMENTS

Four temperature tanks in the greenhouse kept at 15, 20, 25, and 30 C., respectively, were used for these studies. Some fluctuation in temperature occurred, particularly in the 30° tank, but except for short periods the temperatures remained within a degree on either side of the readings indicated for the different tanks.

The soil used was a garden loam which was mixed with sand in the proportion of two parts of soil to one of sand. This soil was steam sterilized and mixed with water, so that it contained approximately thirteen per cent of water by weight. New galvanized iron cans, made especially for the temperature tanks, were then filled with soil. Four cans for each tank were used. The soil in three of these was inoculated, each with a different organism, while the fourth served as a check. The same organisms were used as in the previous temperature

relation studies. The inoculum was mixed in the layer of soil in which the seed was planted. Fifty seeds of Marquis wheat per can were planted, and then the weight of each can was recorded. The soil in the various cans was kept at a constant moisture content by weighing the cans frequently and keeping the weights constant by adding water. Weighings were made every three days and in the intervals graduated quantities of water were added according to the amount of evaporation from the soils in the different tanks. Notes were taken at the end of a month. The results are summarized in Table XIX.

TABLE XIX

RELATION OF SOIL TEMPERATURE TO SEEDLING INJURY CAUSED BY THREE SPECIES OF ROOT ROTTING FUNGI

	Control		F. Moniliforme II	
Temperatures, degrees C.	Seedling survival, per cent	Total dry weight, gms.	Seedling survival, per cent	Total dry weight, gms.
14–16 (15.7)*	98	3.86	78	1.91
19–21 (20.5)	95	3.80	78	2.25
24–26 (25.3)	96	4.29	80	2.27
29–31 (30.3)	92	3.09	66	1.52

	Fusarium, Elegans section (Soil)		Helminthosporium M. I	
Temperatures, degrees C.	Seedling survival, per cent	Total dry weight, gms.	Seedling survival, per cent	Total dry weight, gms.
14–16 (15.7)*	6	0.07	92	2.98
19–21 (20.5)	4	0.01	80	3.14
24–26 (25.3)	2	0.03	60	2.54
29–31 (30.3)	8	0.31	58	1.79

* Mean temperatures.

Altho these experiments should be repeated several times to be conclusive, it so happened that in this series the greatest injury resulted at the temperatures nearest the optimum for the growth of each fungus, i.e., at 30° for *F. moniliforme* and *Helminthosporium M*, and at 25° for *Fusarium sp*. It may be, however, that the effect of temperature on the host was more important than the effect on the fungi. Dickson (18), for instance, obtained similar results in his work on the seedling blight of wheat caused by *Gibberella saubinetii*, but the reverse with the seedling blight of corn caused by the same organism. His experiments and those of Eckerson and Dickson (21) lead to the conclusion that the plants are blighted most severely at temperatures which serve best to predispose the respective plants to disease, i.e., relatively high temperatures for wheat and low temperatures for corn. Soil moisture (18) also was found important. Low soil moistures predisposed wheat seedlings to blight even at low soil temperatures.

EFFECT OF HYDROGEN-ION CONCENTRATION ON GROWTH

Czapek's solution, in small Erlenmeyer flasks, was adjusted to different hydrogen-ion concentrations by adding varying quantities of N/5 HCl and N/20 NaOH. The titration data and method of procedure of Karrer and Webb (40) were followed. The pH readings were first made roughly by the colorimetric method, as given by Clark (14), then final determinations were made by means of a potentiometer. Duplicate flasks of each pH were inoculated with each of the three fungi used in the temperature relation studies. This was accomplished with the Fusarium cultures by introducing 1/10 cc. of a spore suspension of each in sterile distilled water into the different flasks, while with *Helminthosporium M* a small bit of mycelium was introduced into each flask of the series. After *Helminthosporium M* had grown for eleven days, and the *Fusarium spp.* for twenty days at room temperature, the mycelial mats were turned out on weighed filter papers in Büchner filters. These were then dried in a gas oven and the dry weights of the mycelial mats were determined. Results are given in Table XX.

TABLE XX

EFFECT OF HYDROGEN-ION CONCENTRATION ON GROWTH OF THREE ROOT-ROTTING FUNGI ON CZAPEK'S SOLUTION

Initial pH	Dry weights of mycelial mats, mg.		
	Fusarium moniliforme Strain II	Fusarium sp. Elegans section (Soil)	Helminthosporium M Strain 1
1.9	120	14	25
2.8	53	57	317
4.4	57	79	321
5.5	54	92	327
7.0	108	123	277
8.0	106	140	261
8.5	150	118	247
9.3	54	110	206

In general the two species of *Fusarium* grew best on an alkaline medium. The reason for the comparatively heavy growth of *F. moniliforme* on the most acid medium has not been accounted for. The other fungi made scarcely any growth at this extreme acid concentration. *Helminthosporium M* grew well throughout the range except in the most acid concentration. Best growth, however, was obtained on the acid side of the neutral point.

TOXICITY STUDIES

An attempt was made to determine whether the injurious effects on wheat seedlings of certain pathogenic species of *Fusarium* might not be due in part to toxic substances either excreted by the fungi or resulting from their growth on culture medium used in the production of inoculum for soil inoculation studies.

Brandes (10) made somewhat similar experiments and concluded that the wilting of bananas caused by *Fusarium cubense* E. F. Smith amended E. W. Brandes, was not due to the plugging of the vessels by the mycelium but probably resulted from toxic excretions by the fungus. Bisby (4) tested the ability of the filtrates of cultures of several fungi, including *Fusarium spp., Rhizopus, Rhizoctonia,* and *Penicillium,* to wilt leaves of potato, ragweed, and other plants. Wilting occurred in a few hours in most cases but was more rapid in old solutions.

The writer grew different species of Fusarium on sterilized wheat seed and on Uschinsky's solution. Using the former medium, cultures were grown in 250 cc. Erlenmeyer flasks containing equal quantities of the medium. With the latter medium, cultures were grown on 250 cc. of the solution in one-liter Erlenmeyer flasks. When the cultures were well developed they were placed in clean porcelain dishes and frozen over night. To each of the wheat cultures 100 cc. of distilled water was added before freezing. They were then thawed and strained through cheese-cloth. The liquid was pressed out of the solid matter by hand, after which the extracts were filtered through filter paper. Bean plants were cut off under water and placed immediately in small vials of the different extracts and watched for signs of wilting. Wilting was more pronounced and occurred more rapidly with beans than with wheat seedlings. Hence beans were used in these experiments. It was thought that wilting might result from high osmotic pressures. Osmotic pressures of some of the extracts were therefore determined by the freezing point method, using the Beckman apparatus to determine the freezing points, and the table of Harris and Gortner (29) to calculate the osmotic pressures. The results are summarized in Table XXI.

The results indicate that the different pathogenic species of *Fusarium* produce deleterious substances which are not present in the media alone or in distilled water. A few tests with non-pathogenic species, however, gave similar results. Growth of the fungi on wheat seed medium increased the osmotic pressure of the extracts, but the increased osmotic pressure did not seem to cause the wilting, for Uschinsky's solution culture extracts gave higher osmotic pressures but were much less effective in causing wilting, possibly because the toxins were more dilute.

TABLE XXI

EFFECT OF EXTRACTS OF FROZEN FUSARIUM CULTURES ON EXCISED BEAN PLANTS

Fungus	Medium	O.P. of extract in atmospheres	Age of culture, days	Notes on wilting
Fusarium moniliforme	Wheat seed	2.676	42	2½ hours, moderate wilting
Gibberella saubinetii	"	1.978	42	2½ hours, pronounced wilting
Fusarium sp. (Pathogenic)	"	4.435	42	do
Check (Wheat seed extract)	"	0.109	42	2½ hours, none
Check (Distilled water)	..	....	..	do
Fusarium moniliforme	Wheat seed	....	30	2 hours, moderate wilting. 12 hours, pronounced wilting
Fusarium sp. (Non-pathogenic)	"	....	30	2 hours, moderate wilting. 12 hours, pronounced wilting
Check (Distilled water)	..	....	30	2 hours, none, 12 hours, none
Fusarium moniliforme	Wheat seed	....	37	2 hours, moderate wilting. 24 hours, pronounced wilting
Gibberella saubinetii	"	4.279	37	2 hours, moderate wilting. 24 hours, pronounced wilting
Fusarium sp. (Pathogenic)	"	4.399	37	2 hours, moderate wilting. 24 hours, pronounced wilting
Check (Distilled water)	..	....	..	2 hours, none, 24 hours, none
Fusarium moniliforme	Uschinsky's solution	6.049	28	2 hours, none, 24 hours, moderate wilting
Gibberella saubinetii	"	5.904	28	2 hours, none, 24 hours, moderate wilting
Fusarium sp. (Pathogenic)	"	7.265	28	2 hours, none, 24 hours, moderate wilting
Check (Uschinsky's solution)	"	6.049	..	2 hours, none, 24 hours, none
Check (Distilled water)	..	....	..	2 hours, none, 24 hours, none

SOIL STERILIZATION EXPERIMENTS

If poor growth and reduced yields of wheat on certain soils are due to root-rotting fungi, then thoro sterilization of seed and soil should restore productiveness. Field observations on continuously cropped and rotated wheat plots of the agronomy division,[12] University Farm, St. Paul, were made. Most of the stunted and unthrifty plants on the continuous-wheat plots were affected with root- and foot-rot, while there was a much lower percentage of diseased plants on the rotation plots. This is indicated by Table XXII, in which fifty plants selected at random on July 17, 1922, from a continuous wheat

[12] The writer is indebted to Professor A. C. Arny for soil samples and permission to use data from these plots.

plot, are compared with fifty from a five-year rotation plot. The plants were brought to the laboratory and washed free of soil and then examined. The notes cover only a very small proportion of the root systems, as only the upper 3 to 4 inches was examined.

TABLE XXII

Comparison of Amount of Foot- and Root-Rot on Marquis Wheat Grown Continuously and in Rotation

Cropping system	Foot-rot	Root-rot		Av. No. of stools per plant	Mean height, cms.	Total dry weight, gms.
	Per cent of plants diseased	Per cent of roots diseased	Severity*			
Continuous wheat since 1893	58	50	43	1.1	52	22.5
Five-year rotation (wheat, pasture, clover, oats, corn)	20	22	24	1.8	122	181.0

* Severity was estimated arbitrarily by allowing 100 points for very heavy infection, 75 for heavy, 50 for moderate, 25 for light, 5 for trace, and 0 for normal roots. The estimates for the 50 plants were then totalled, and the percentage which this figure represented of the total possible severity (5000) was calculated.

Plate X, Figure 2 (A and C) shows the comparative growth of wheat on these two plots in the field; and Figure 1, the beneficial effect of steam sterilization on the growth of wheat seedlings on the continuous-wheat plot soil. Steam sterilization not only controlled root-rot but also increased the vigor of the seedlings very markedly. It is realized, however, that soil sterilization with steam produces marked changes in the chemical and biological content of the soil, as pointed out by Johnson (39), Russel (64), and others. How much of the increased vigor of the wheat seedlings is due to root-rot control and how much to other changes resulting from sterilization is difficult to say, but the former undoubtedly is important. In subsequent soil sterilization experiments, formalin was used as a disinfectant instead of steam, as it was considered that it would cause less pronounced changes in the composition of the soil. A 1 to 50 formalin solution as recommended by Johnson (39) was used.

Experiments were undertaken to determine the relative effectiveness of soil sterilization on fertilized and unfertilized soils, some of which had borne wheat continuously for several years, others which had never been cropped to wheat, and others on which wheat had been sown only once in every three or five years. Fertilizers were applied in order to overcome any plant food deficiency in these soils. A liberal quantity of lime was used in order to render the soils somewhat alkaline. The following fertilizer was applied to two of the series, one of which was also sterilized: Ground limestone at the rate of 3 tons

per acre; treble superphosphate, 250 pounds per acre; potassium chloride, 250 pounds per acre; nitrate of soda, 125 pounds per acre; and dried blood, 125 pounds per acre. Sterilization was accomplished by standing pots of soil in a 1 to 50 formaldehyde solution in closed chambers over night. Unsterilized series were kept in water for the same length of time. The soils were then allowed to dry until the fumes of formaldehyde disappeared from the sterilized soil. Two-quart earthenware jars were then filled with the different soils and thirty seeds of sterilized Marquis wheat per pot were planted. The seedlings were later thinned to twenty per pot in order to have a uniform number of plants in the different pots. All pots received equal amounts of water and their positions in the greenhouses were changed at intervals in order to give them similar conditions as regards light and other factors. The results of these experiments are summarized in Tables XXIII and XXIV.

Sterilization of the unfertilized soils in the experiment, summarized in Table XXIII, caused an increased growth over the untreated soil in every case, but this was most pronounced in No. 1. Sterilization and fertilization combined gave the maximum growth in No. 2 and No. 3 but the results in No. 1 were inconclusive, as the tops of the plants were partly destroyed by mice during the experiment. The application of fertilizers alone, as judged by the total dry weight of the tops, was somewhat less effective in increasing growth than sterilization in No. 1 and No. 2, but was more effective on the virgin soil.

TABLE XXIII

Effect of Formaldehyde Sterilization of Fertilized and Unfertilized Wheat Soils on the Growth of Marquis Wheat

No.*	Soil	Growth Measurements after 32 days	Soil treatment: No treatment	Sterilized	Fertilized	Fertilized and sterilized
1	Continuous-wheat soil	Mean height, cms.	39.70	52.30	53.20	53.10†
		Dry weight, gms.	1.82	2.78	2.62	2.30†
2	Five-year rotation soil	Mean height, cms.	49.30	53.30	55.20	55.50
		Dry weight, gms.	2.86	3.07	2.51	3.36
3	Virgin soil	Mean height, cms.	44.30	48.60	50.00	53.90
		Dry weight, gms.	1.94	2.11	2.40	2.71

* 1 In wheat since 1893.
2 Wheat, clover, pasture, oats, corn.
3 From forestry plantation near the continuous-wheat plot.
† Tops attacked by mice.

Another similar experiment was made. In this experiment, however, a three-year rotation soil replaced the virgin soil and careful notes were taken on the relative severity of root- and foot-rots in the different series. The reaction of the soil was also determined at the conclusion of the experiment. The figures, except those for dry weights in Table XXIV, represent average results from four pots for each of the different treatments.

TABLE XXIV

Effect of Formaldehyde Sterilization of Fertilized and Unfertilized Wheat Soils on the Growth of Marquis Wheat, on the Development of Foot- and Root-Rots, and on the Hydrogen-ion Concentration of the Soil

Soil	Soil treatment	Growth measurements		Disease notes				
				Foot-rot		Root rot		
		Mean height, cms.	Total dry weight, gms.	No. of plants	Degree*	No. of plants	Degree*	pH of soil
Continuous-wheat soil	a. No treatment	17.15	3.17	64	36.2	60	24.5	6.2
	b. Sterilized	22.37	4.14	0	0.0	8	0.5	6.1
	c. Fertilized	20.17	3.32	56	17.2	48	10.5	7.7
	d. Sterilized and fertilized	26.50	4.17	12	3.0	0	0.0	7.3
Three-year rotation soil	a. No treatment	18.02	2.79	44	20.2	36	7.2	6.3
	b. Sterilized	22.77	3.91	4	0.2	12	0.7	6.0
	c. Fertilized	22.87	3.78	52	19.5	36	7.2	7.7
	d. Sterilized and fertilized	25.47	4.08	0	0.0	4	0.2	7.1
Five-year rotation soil	a. No treatment	18.55	2.93	40	13.5	64	16.7	6.0
	b. Sterilized	27.57	4.33	0	0.0	4	0.2	5.9
	c. Fertilized	21.57	3.30	48	17.2	36	4.2	7.3
	d. Sterilized and fertilized	27.90	4.48	12	1.7	40	4.5	6.9

* Estimated as in Table XXII.

In this experiment there was very little difference in growth between seedlings on the different soils in the untreated series. In the field, however, there were marked differences on these three soils (Plate X, Fig. 2). Soil sterilization with formalin not only reduced the amount of root- and foot-rot in all the soils to a negligible amount, but also caused increased growth of the plants in every case and seemed to increase slightly the acidity of the soil. It is possible that in the field the continuous-wheat soil would be benefited much more than the rotation soils by soil sterilization. Soil fertilization with a liberal application of lime changed the soil reactions from acid to alkaline but did not materially reduce the amount of root-rot in this experiment. It did result in increased growth but was not as beneficial as soil sterilization. On soils which had been sterilized and fertilized, the plants grew best, and there was little foot- and root-rot. While it is obviously not possible to sterilize soil in any extensive way in the field, it is possible to practise crop rotation and in this way, perhaps, accomplish the equivalent of a partial sterilization. This, accompanied by the application of suitable fertilizers, particularly phosphorus and lime to soils requiring these chemicals, should materially aid in controlling root-rots.

OVERWINTERING STUDIES

One of the most important points to determine concerning the life history of root-rotting fungi is their method of overwintering. It is generally known that several overwinter in or on the seed, and it is frequently said that some also overwinter in the soil, on old stubble and other debris, but many such statements seem to be based mostly on observational evidence. Thus, with respect to *Fusarium moniliforme,* Sheldon (69) states that it probably overwinters in the soil; Valleau (84) says that "no evidence has yet been obtained as to the ability of *F. moniliforme* to live over winter in the soil"; while Manns and Adams (48) believe that certain corn parasites, including *F. moniliforme,* live over from season to season on cornstalks, roots, and ears lying in the field.

The writer selected cultures for overwintering studies and grew them on sterilized wheat seed in small flasks, as well as on sterilized wheat straw in large test-tubes. On both media the fungi grew well, and most of them sporulated abundantly. Several 4-inch pots of soil were sterilized by steam, and a wheat seed culture of each fungus was added to each pot, about one inch below the surface of the soil. On November 9, 1922, these pots were placed outside and partly sunk in the soil. The straw cultures were placed in the refrigerator on December 2, and on December 10 were taken out and bound separately

in small packets, using ordinary window screening as a covering. They were then stuck in the surface of the soil, outside, near the greenhouse. On April 4 and 5, 1923, viability tests were made.

An attempt was made to recover pure cultures of the different fungi from the original, over-wintered material; spore germination tests were made to see if the over-wintered spores were still viable. The procedure was as follows: In making re-isolations from the soil, a bit of the original culture of each fungus was removed from the soil with a sterilized platinum needle and transferred to a tube of sterile distilled water. After being washed in this for a few minutes, it was transferred to the center of a plate of acidified potato dextrose agar. The washings, as a rule, contained an abundance of spores, and these were tested for germination in syracuse dishes. On the other hand, pieces of the original straw cultures were scraped with a sterilized knife and the scrapings placed on the surface of sterile distilled water in syracuse dishes for spore germination studies. Sections of straw from the different cultures were also placed on moist, sterilized filter paper, in sterilized petri dishes. If, after several days, the fungi were sporulating on the filter paper some distance from the straw, the trials were considered positive.

The results are summarized in Table XXV and are rather interesting. Practically pure cultures of the various fungi were recovered in every instance from the media overwintered in the soil. Moreover, typical spores were produced by all the colonies except those of *Gibberella saubinetii* and *Fusarium sp.*, but the colonies of these fungi were readily recognized, so that there was practically no doubt of their identity. The germinating spores of the different species of *Helminthosporium* and those of *Stemphylium* could, of course, not be mistaken, but those of the *Fusarium spp.* were less readily identified. The spores of three of the *Helminthosporia* germinated, but those of *Helminthosporium X* did not. Christensen (13) found that the mycelium of *H. sativum* over-wintered in the open at St. Paul, but none of the spores germinated. In these tests, however, a comparatively high percentage of the spores of *H. sativum* over-wintered. The tests with the straw cultures gave similar results except that the recovery of *G. saubinetii* and *Fusarium sp.* was not conclusive, as they were not readily identified on the filter paper medium. *Helminthosporium X* and *Stemphylium parasiticum* were not included in these tests.

In general, the results indicate that the mycelium of all the fungi tested and the spores of some readily over-winter on debris in the soil or on its surface.

TABLE XXV

SUMMARY OF OVER-WINTERING STUDIES

Fungus	Kept over winter in soil		Kept over winter on straw	
	Re-isolation in culture	Spore germination, per cent	Re-isolation in culture	Spore germination, per cent
Fusarium moniliforme Strain I	+*	(?)ab†	+*	(?)ab†
Fusarium moniliforme Strain II	+		+	
Gibberella saubinetii	+	(?)ab†	?	
Fusarium sp. (Elegans section).....	+		?	
Helminthosporium sativum	+	64	+	84
Helminthosporium N	+	0		
Helminthosporium M Strain I	+	96	+	50
Helminthosporium M Strain IV	+		+	
Helminthosporium pedicellatum	+	4	+	16
Stemphylium parasiticum	+	100		

* Typical cultures of the fungus were recovered.

† ab=abundant.

CONCLUSIONS

Altho most of the fungi tested were not virulent root parasites, those that were parasitic were sufficiently destructive and widely distributed that the results lend further support to Bolley's contention that such fungi contribute materially to the deterioration of wheat yields. While we are not justified in saying that root-rotting fungi are the only cause of reduced wheat yields on land more or less constantly cropped to wheat, we can at least say that they constitute an important contributing factor in this reduction.

The old theory of De Candolle that toxic excreta of plant roots are responsible for unproductiveness has little evidence to support it according to Russel (64, 65) and Livingston (46), but more recent evidence on soil toxins has thrown new light on the cause of unproductive soils (5, 33, 83). The presence of available deleterious aluminum and iron salts in certain soils may be important in predisposing wheat plants to root-rot, as has been shown by Hoffer for corn. The precipitation of these injurious salts by the application of phosphates and lime may explain in part the beneficial action (8) of these fertilizers on certain "wheat-sick" soils.

The fact that root-rotting organisms such as *Gibberella saubinetii, Helminthosporium sativum*, and *Fusarium moniliforme,* live over winter in and on the seed and as saprophytes on plant remains in and on the surface of the soil, and that they are intercrop parasites, make the control of root-rots a difficult problem. Dickson (18), Dosdall (19), Hoffer (83), and others have pointed out, however, that maximum damage from these pathogenes results when conditions for host development are unfavorable. Until resistant varieties can be developed, control measures must consist in providing as nearly optimum conditions as possible for host development, combined with such general preventive measures as seed treatment, crop rotation, and field sanitation.

SUMMARY

1. A study was made of the relation of fungi to root-rots of wheat.

2. Cultures of fungi were isolated for pathogenicity studies from many sources, but chiefly from wheat seeds, from "wheat sick" soil, and from the underground parts of the wheat plant.

3. Species of fungi representing between fifteen and twenty genera were isolated from wheat seeds. *Alternaria* was the most common fungus on the seeds. Several pathogenic species of fungi were obtained.

4. The fungi of black-pointed seeds were studied in particular. *Helminthosporium sativum* was the most common cause of black-point. It was proved by inoculation studies, however, that two other members of this genus, designated in the text as *Helminthosporium N* and *Helminthosporium M*, as well as *Stemphylium parasiticum* can also cause black-point.

5. Most of the fungi obtained directly from the soil were non-pathogenic, but a few pathogenes were isolated.

6. Several pathogenes were isolated from the underground parts of the wheat plant, and a few were obtained from other sources.

7. In preliminary pathogenicity studies in the laboratory, 76 out of 301 cultures tested were marked pathogenic, but many of these were duplicates of the same species.

8. Extensive pathogenicity studies in the greenhouse demonstrated that the important pathogenes of wheat roots encountered in these studies belong to the genera *Fusarium* and *Helminthosporium*. Some species of these genera, however, were also non-pathogenic or weak pathogenes. Certain strains of *Alternaria*, *Phoma*, *Acrostalagmus*, and *Aspergillus* penetrated the roots or caused deformities of them, but it was not considered that they would be destructive in the field. Species representing twenty-one other genera were non-pathogenic.

9. Among the *Fusaria*, *F. graminearum* (*Gibberella saubinetii*) was the most virulent pathogene of any tested. Certain strains of *Fusarium moniliforme* and two species of the Elegans section, tho less destructive, were also decidedly parasitic on wheat roots. The strain of *F. culmorum* used, apparently had lost its virulence. The other *Fusaria* tested, including *F. lini*, *F. betae*, and numerous unidentified species, were non-pathogenic or only slightly pathogenic.

10. *Fusarium moniliforme* in addition to wheat also attacked barley, rye, oats, and corn. Four strains of the fungus were studied. One of these, Strain IV, was much less destructive to wheat seedlings than the other three. It could also be distinguished from them by its reaction on different media. The other strains were much alike, altho Strain I differed slightly from Strains II and III.

11. *Helminthosporium sativum* was in general the most common and the most destructive species of *Helminthosporium* found. At least three other species of *Helminthosporium* were isolated from wheat.

12. The species of *Helminthosporium* designated *Helminthosporium N* has conidia which are significantly narrower than those of *H. sativum*. This organism produced symptoms similar to those caused by *H. sativum* on different parts of the wheat plant, including the roots, but it was much less virulent.

13. Several similar small-spored *Helminthosporia* were isolated. Four strains, three from wheat seed and one from a millet leaf, were studied. Two of these were destructive pathogenes, and attacked different parts of the wheat plant besides the roots, while the other two were much weaker pathogenes. The four strains were readily distinguished by cultural characters and differences in pathogenicity. Significant differences in the morphology of their spores were shown by biometrical studies. At least three elementary species, or perhaps true species, were represented.

14. A new species of *Helminthosporium, H. pedicellatum n sp.*, is described. It was isolated from diseased wheat roots and when these were artificially inoculated the cortex was penetrated but the injury was slight.

15. Most fungi attacked the piliferous and cortical regions of the root only. The structure and arrangement of the endodermal cells seemed to constitute an effective barrier against the entrance of these fungi into the stele. However, several species of *Fusarium* attacked the central cylinder primarily, and caused disintegration of the cell walls.

16. The seedling stage of the wheat plant is undoubtedly a critical period in its life from the standpoint of root-rots. The seminal roots seem particularly susceptible to attack while the adventitious roots seem somewhat more resistant.

17. *Fusarium moniliforme* Strain II and *Helminthosporium M*, Strain I, grew best at about 30° C., whereas *Fusarium sp.* (Elegans section) had a slightly lower optimum. Two strains of *F. moniliforme*, one from seed and the other from soil, showed approximately the same reaction to different temperatures.

18. *F. moniliforme* Strain II and *Helminthosporium M* Strain I, and *Fusarium sp.* (Elegans section) in one experiment were more destructive to wheat seedlings at a soil temperature nearest their respective optimum temperatures for vegetative growth, than at other temperatures between 15 and 30° C.

19. On Czapek's solution, *F. moniliforme* Strain II and *Fusarium sp.* (Elegans section) grew best when the medium was somewhat alkaline (pH 7 to 8.5), while *Helminthosporium M* Strain I, grew best on the acid side of the neutral point (pH 2.8 to 5.5).

20. Extracts of frozen cultures of pathogenic and non-pathogenic species of *Fusarium* caused wilting of excised bean plants, whereas no wilting occurred during the experiment in the medium alone (Uschinsky's solution) or in extracts of the medium (wheat seed) or in distilled water. Wilting apparently was not due to high osmotic pressure of the extracts.

21. Formaldehyde disinfection of soils which had been cropped to wheat for different periods of time caused increased growth of wheat seedlings over those on untreated soils in all cases. In one experiment this increase was most pronounced on a continuous wheat soil, as compared with a virgin soil and a five-year rotation soil. Increased growth was accompanied by root-rot control. The application of fertilizers, including the liberal use of lime, also caused marked increase in growth but was less effective than soil sterilization in controlling root-rot. Wheat plants grew best on soil which had been disinfected and fertilized. In farming practice the rotation of unrelated crops probably offers the best substitute for soil sterilization.

22. Cultures of *Fusarium moniliforme, Gibberella saubinetii, Fusarium sp.* (Elegans section), *Helminthosporium sativum, Helminthosporium N, Helminthosporium M, Helminthosporium pedicellatum,* and *Stemphylium parasiticum* were recovered from inoculum of each on wheat seed over-wintered under the soil surface from November 9, 1922, to April 4 and 5, 1923, at St. Paul. The over-wintered spores of *H. sativum, Helminthosporium M, H. pedicellatum,* and *Stemphylium parasiticum* germinated. Several of these fungi also over-wintered on straw kept on the surface of the soil.

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EXPLANATION OF PLATES

Plate I

Effect of Inoculating Marquis Wheat Seedlings with *Fusarium spp.* Illustrating Method Used in Preliminary Pathogenicity Studies

Photograph taken one month after inoculation

1. Inoculated with *Fusarium moniliforme* Strain II from the soil
2. Inoculated with *Fusarium moniliforme* Strain I from wheat seed
3. Inoculated with *Fusarium graminearum* (*Gibberella saubinetii*) from wheat roots
4. Uninoculated—Control
5. Inoculated with *Fusarium graminearum* from wheat seed
6. Inoculated with *Fusarium graminearum* from wheat roots
7. Inoculated with *Fusarium graminearum* from wheat roots

Plate II

Fig. 1. Black-Point of Wheat Caused by Different Fungi

A. Marquis seeds from head inoculated with *Helminthosporium sativum*

B. Left—Marquis seeds from head inoculated with *Helminthosporium M*
Right—Kubanka seeds from head inoculated with *Helminthosporium M*

C. Marquis seeds from head inoculated with *Stemphylium parasiticum*

The two upper rows of kernels are from inoculated heads and show embryo discoloration. The two lower rows are from uninoculated heads and show normal embryos.

Fig. 2. Three minute black sclerotium-like bodies on wheat roots collected in the field at St. Paul

Plate III

Effect on Growth of Marquis Wheat Seedlings of Soil Inoculation with *Fusarium moniliforme*

Fig. 1. Comparison of the relative virulence of Strains IV and III. All pots were planted at the same time, and all inoculated pots received approximately equal quantities of inoculum

A. Soil inoculated with Strain IV

B. Soil uninoculated—no medium added to soil—control

C. Soil inoculated with Strain III

Fig. 2. Typical series used in pathogenicity tests in the greenhouse

A. Soil uninoculated—culture medium added to soil—control

B. Soil inoculated with *F. moniliforme* Strain I

Plate IV

Effect on Roots and Tops of Marquis Wheat Seedlings of Soil Inoculation with Two Strains of *Fusarium moniliforme*

Photograph taken one month after planting

1. Seedlings from soil inoculated with Strain I
2. Seedlings from uninoculated soil (medium added)—control
3. Seedlings from soil inoculated with Strain II

Plate V

Effect on Different Cereals of Soil Inoculation with *Fusarium moniliforme* Strain I

Fig. 1. A. Effect on wheat—left to right—control (no medium added to soil) two pots of inoculated soil, control (medium added to soil)

B. Effect on rye—left to right—as in A

Fig. 2. A. Effect on barley—left to right—as in Fig. 1, A

B. Effect on oats—left to right—as in Fig. 1, A

Fig. 3. A. Effect on field corn—left to right—control (no medium added to soil), control (medium added to soil), two pots of inoculated soil

B. Effect on sweet corn—left to right—two pots of inoculated soil, control (no medium added), control (medium added to soil)

Fig. 4. Comparative virulence of different fungi on Minhardi wheat

The different pots received approximately equal quantities of inoculum and were each sown with twenty seeds of Minhardi wheat. Photograph taken 12 days after planting.

1. Soil inoculated with *Gibberella saubinetii* from wheat seed.
2. Soil inoculated with *Fusarium sp.*, Elegans section, from wheat roots
3. Soil inoculated with *Gibberella saubinetii* from wheat roots
4. Soil inoculated with *Fusarium moniliforme* Strain II from the soil
5. Soil inoculated with *Fusarium moniliforme* Strain I from wheat seed
6. Soil inoculated with *Helminthosporium M* Strain IV from millet leaf
7. Soil inoculated with *Helminthosporium sativum* from wheat seed
8. Soil inoculated with *Helminthosporium pedicellatum* from wheat roots
9. Soil inoculated with *Stemphylium parasiticum* from wheat seed
10. Uninoculated—control

Plate VI

Comparison of Cultural Characters of Strains I, II, III, and IV of *Helminthosporium M* on Three Different Media: Oat Agar (left), Czapek's Agar (center), and Potato Dextrose Agar (right)

Plate VII

Fig. 1. Effect of soil inoculation with *Helminthosporium M* Strain I on tops and roots of Marquis wheat seedlings

A. Grown in inoculated soil

B. Grown in uninoculated soil

Fig. 2. Effect of temperature on vegetative growth of two species of *Fusarium* on potato dextrose agar

A. *Fusarium sp.* (Elegans section)

B. *Fusarium moniliforme*

Plate VIII

Fig. 1. Effect of soil inoculation with four strains of *Helminthosporium M*, on Kanred wheat. All pots were planted at the same time and all the inoculated pots received approximately equal quantities of inoculum.
A. Left, soil inoculated with Strain II
Right, soil inoculated with Strain III
B. Soil uninoculated—control
C. Left, soil inoculated with Strain IV
Right, soil inoculated with Strain I

Fig. 2. Effect of soil inoculation with *Acrostalagmus sp.* on Marquis wheat roots. All three seedlings were taken from the same pot. The roots of the two outer seedlings showed marked stunting, hypertrophy, and other deformities. Their roots were also rotted, but chiefly at the tips. The roots of the central seedling, however, were more typical of the majority of the plants in the inoculated pots.

Plate IX

Photomicrographs of Cross-Sections of Seminal Roots of Marquis Wheat Attacked by Different Fungi

Fig. 1. Cross-section of a root, showing the mycelium of *Phoma sp.* confined to the cortex

Fig. 2. Cross-section of another root attacked by *Phoma sp.*, showing the abundant production of chlamydospores by the mycelium in the cortex

Figs. 3 and 4. Sections of two seminal roots, showing the invasion of the mycelium of *Helminthosporium M* as far as the endodermis. A few of the endodermal cells have been entered but the stele has not been attacked.

Fig. 5. Cross-section of a root attacked by *Fusarium moniliforme.* The mycelium of the fungus is very plentiful in the cells of the central cylinder and the walls of these cells have commenced to decay. The cortex is much less severely attacked.

Fig. 6. Cross-section of another root attacked by *Fusarium moniliforme.* The stele has disintegrated and the cavity is filled with mycelium. In this stage of decay the cortex is also thoroly invaded with mycelium but the cells have not entirely broken down.

Plate X

Fig. 1. Effect of steam sterilization of a "wheat sick" soil on the growth of Marquis wheat seedlings
This soil had borne wheat continuously since 1893 except for 1915 and 1916, when it was planted to corn.
A. Seedlings grown on steam sterilized soil
B. Seedlings grown on unsterilized soil
Ten seeds were planted in both A and B.

Fig. 2. Relative growth of Marquis wheat under different systems of rotation
A. Fifty plants selected at random from a five-year rotation plot
B. Fifty plants selected at random from a three-year rotation plot
C. Fifty plants selected at random from a plot which had been in wheat continuously since 1893, except in 1915 and 1916.

Plate XI

Conidia and Conidiophores of Different Species of Helminthosporium Isolated from Wheat

All drawings were made with the aid of a camera lucida, to the scale indicated on the plate.

1. Three conidia of *Helminthosporium sativum*
2. Four conidia of *Helminthosporium M* Strain I
3. Three conidia of *Helminthosporium N* showing one still attached to a conidiophore
4. Conidia, germinating conidia, and conidiophores of *Helminthosporium pedicellatum*

Plate XII

Drawings of *Helminthosporium M*, Strain I and *Stemphylium parasiticum* Illustrating Conidial and Conidiophore Germination

All drawings were made with the aid of a camera lucida, to the scales indicated on the plate.

1. Germinating conidium of *Helminthosporium M*, showing rapidity of germ-tube growth. The figures are in minutes. The first germ-tube was visible in fifty minutes.
2. Two germinating conidia of *Helminthosporium M*, showing typical germination from the end cells of the spores
3. A conidium of *Helminthosporium M*, showing one germ-tube coming from a central cell of the spore
4. Germinating conidiophore of *Helminthosporium M*
5. Conidiophores and attached conidia of *Helminthosporium M*
6. Germinating conidia of *Stemphylium parasiticum* from wheat
7. Conidia and conidiophores of *Stemphylium parasiticum* from wheat
8. Germinating conidiophores of *Stemphylium parasiticum* from wheat

PLATE I

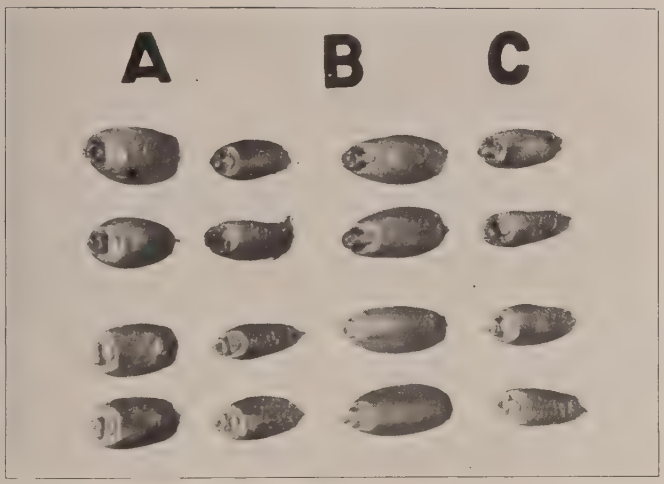


Figure 1

Figure 2

PLATE III

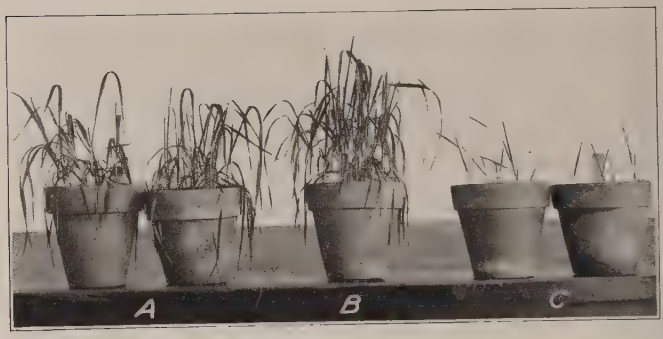


Figure 1

Figure 2

PLATE IV

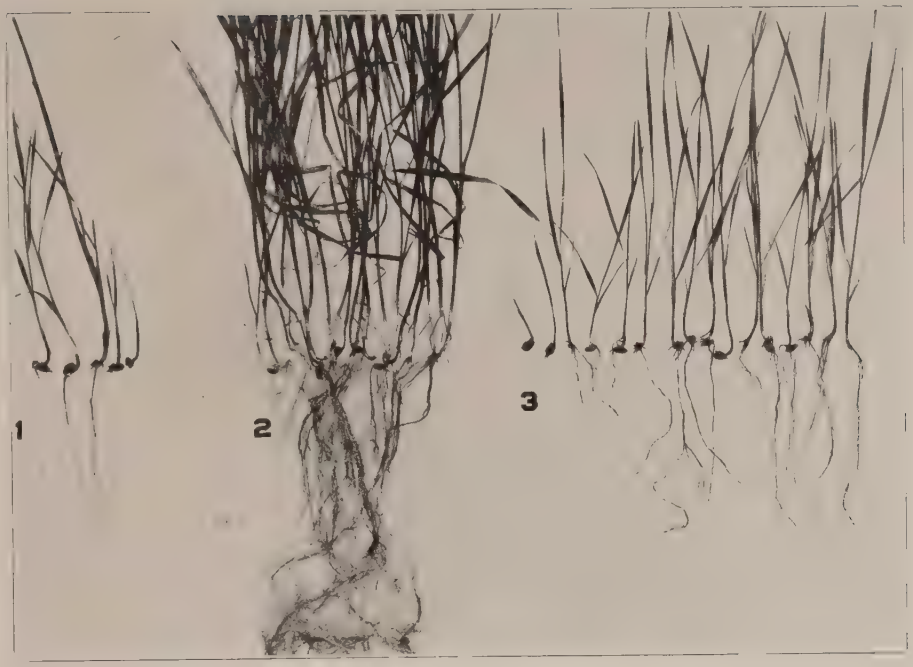


PLATE V

Figure 1

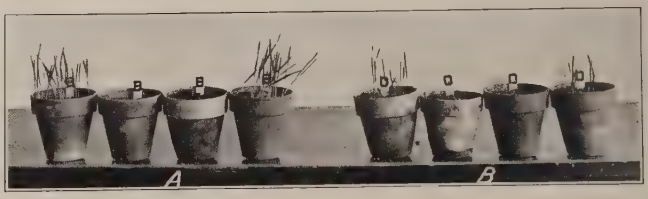


Figure 2

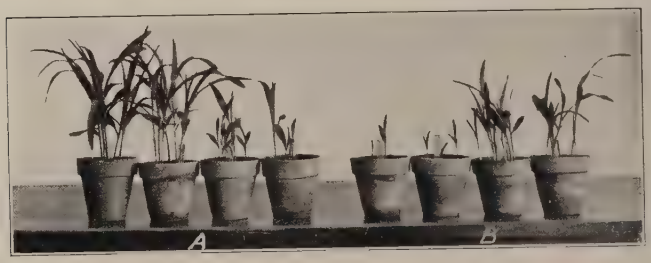


Figure 3

Figure 4

PLATE VI

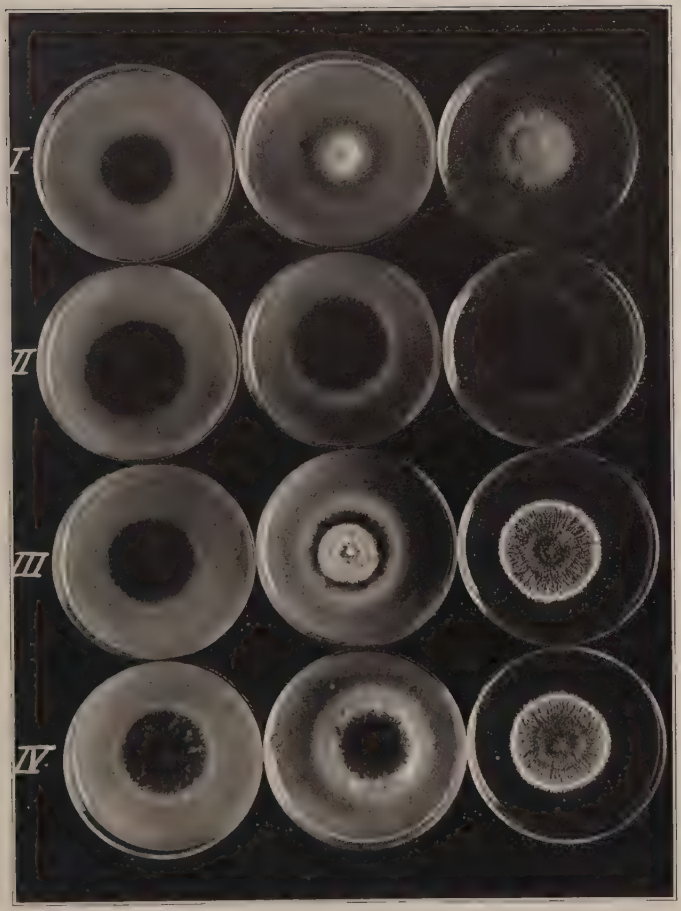


Figure 1

Figure 2

PLATE VIII

Figure 1

Figure 2

PLATE IX

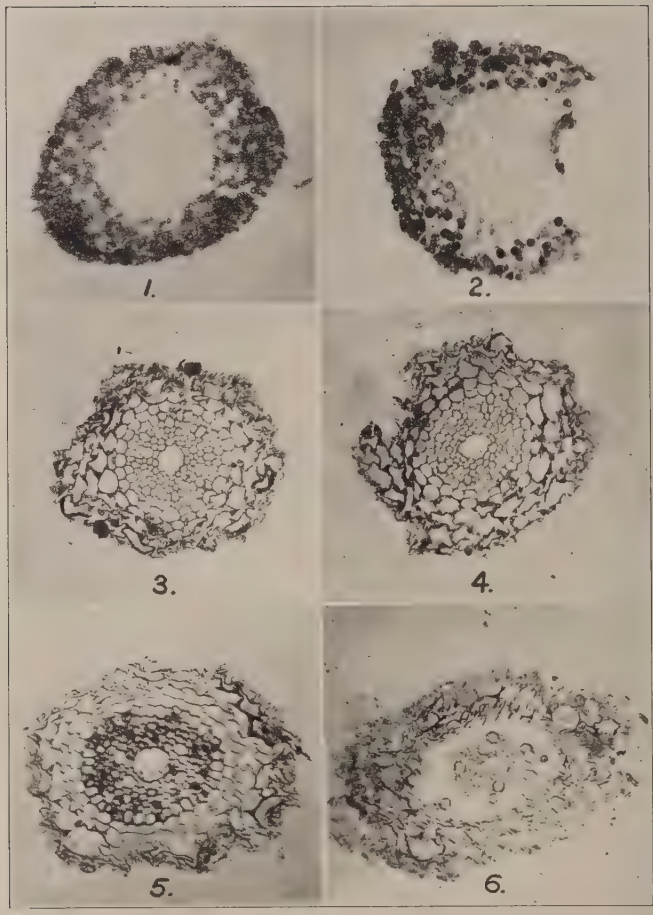


Figure 1

Figure 2

PLATE XI

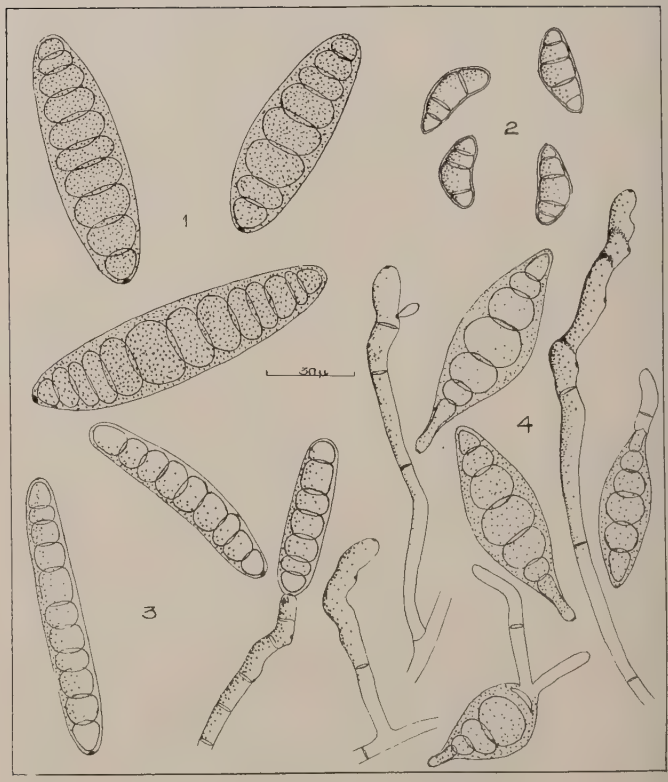
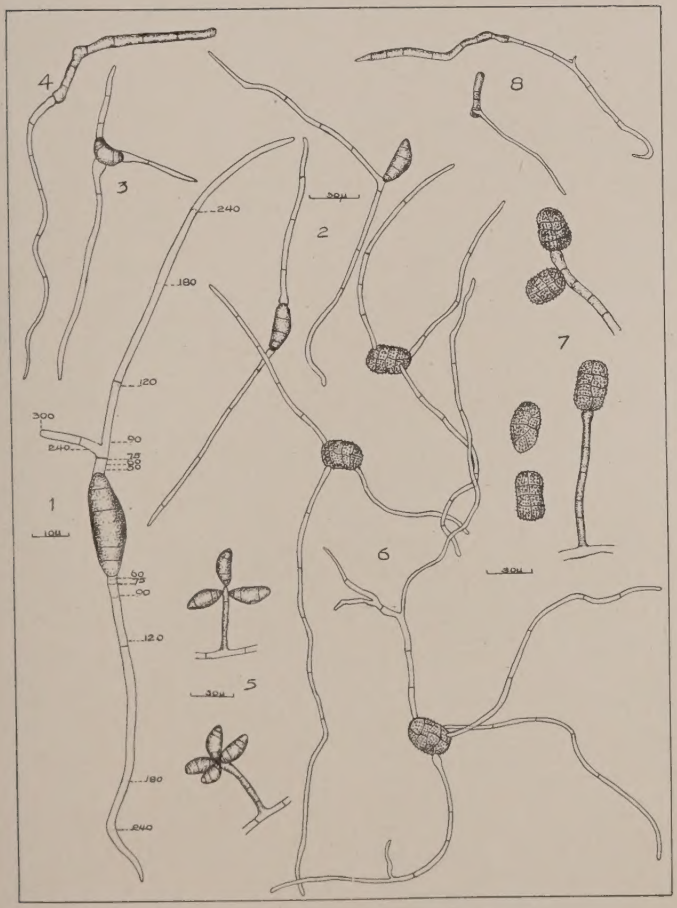


PLATE XII



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